STUDY ON INTERACTIVE EFFECT OF HEAT TREATMENT AND ENZYMATIC ACTIVITIES IN MATURE COCONUT WATER UPON STORAGE

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By

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ABSTRACT

STUDY ON INTERACTIVE EFFECT OF HEAT TREATMENT AND ENZYMATIC ACTIVITIES IN MATURE COCONUT WATER UPON STORAGE

Yew Yee Syuan

Mature coconut water (MCW), though nutritionally valuable, is often discarded during coconut milk production. Factors such as heat treatment, sugar addition and enzymatic activity could lead to browning of MCW during processing and storage. Phenolic compounds in coconut water serve as substrate for enzymatic browning. Chitin, a renewable biopolymer has been widely applied in wastewater treatment and white wine production to remove phenolic compounds. However, research on its application in coconut water is limited. This study examined the effects of heat treatments (water bath, W; pressure cooking, P) and their combinations with chitin (CW, CP) on enzymatic browning in MCW. Changes in total phenolic content (TPC), browning index (BI), polyphenol oxidase (PPO) activity and peroxidase (POD) activity were monitored over 12 days. POD were absent in all samples, whereas PPO activity was highest in untreated MCW (control) and progressively decreased in W (64.00 ± 0.00 U/mL), CW (40.00 ± 0.00 U/mL) and was undetectable in P and CP (0.00 ± 0.00 U/mL) by Day 12. W and CW did not inhibit the browning (a progressive increase of BI from Day 0 to Day 12 storage). In contrast, P and CP treatments significantly suppressed browning in MCW, with no

significant changes in BI between Day 0 and Day 12. These findings suggest that pressure-cooking is more effective in controlling browning, with no notable difference between P and CP. Chitin application in MCW requires further study, as its effectiveness appears highly dependent on the surrounding chemical environment. Overall, the combination of chitin and heat treatment showed similar effects to heat treatment alone. Despite these challenges, MCW retains potential for commercialization as a sports drink alternative due to its beneficial properties, although further research is needed to develop a product that is both appealing and nutritious.

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Last but not least, I sincerely appreciate the love, support and encouragement provided by my family and friends throughout my final year project. Their support has truly been what keeps me going.

DECLARATION

I hereby declare that this final year project report entitled **"STUDY ON INTERACTIVE EFFECT OF HEAT TREATMENT AND ENZYMATIC ACTIVITIES IN MATURE COCONUT WATER UPON STORAGE"** 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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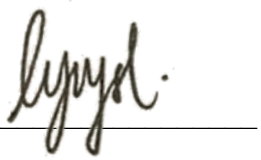

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Date: 7 Oct 2025

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LIST OF ABBREVIATIONS

a*	Redness
ANOVA	Analysis of variance
Aw	Water activity
b*	Yellowness
BI	Browning Index
CFU/mL	Colony-forming units per milliliter
CP	Chitin plus pressure-cook treatment
CW	Chitin plus water bath treatment
E. coli	*Escherichia coli*
F-C	Folin-Ciocalteu
GAE	Gallic acid equivalents
HRP	Horseradish peroxidase
K. pneumoniae	*Klebsiella pneumoniae*
L*	Lightness
MCW	Mature coconut water
NaCl	Sodium chloride
P	Pressure-cook treatment alone
PCA	Plate Count Agar
POD	Peroxidase
PPO	Polyphenol oxidase
p-value	Probability
rpm	Revolutions per minute

SD	Standard deviation
SET	Single electron transfer
TPC	Total Phenolic Content
Tukey’s HSD	Tukey’s honest significant difference
UV	Ultraviolet
VBNC	Viable but non-culturable
W	Water bath treatment alone

CHAPTER 1

INTRODUCTION

1.1 Background

Coconut water, extracted from the nut of young and properly matured coconuts (*Cocos nucifera*), is a widely consumed beverage in tropical countries (Juli, et al., 2023). It is a low-calorie beverage and has been recognised for its numerous health and therapeutic benefits, including alleviating diarrhea, dehydration, fatigue, digestive issues and heatstroke (Sanganamoni, Mahanti and Rao, 2018). Moreover, coconut water also contains phenolic compounds commonly found in fruits and vegetables, which contribute to antioxidant properties (Juli, et al., 2023). For instance, catechin and epicatechin present in coconut water exhibit antimicrobial, antioxidant and anticancer activities. Furthermore, due to its natural electrolyte content, coconut water serves as an excellent alternative to conventional sports drink for rehydration (Juli, et al., 2023).

Mature coconut water (MCW), on the other hand is often discarded as industrial waste during coconut milk production since only the flesh is utilised for processing. This practice may be attributed to its lower palatability, as the sweetness decrease and a slightly salty and sour taste develops as the coconut matures. Typically, coconuts are considered as mature at around 9 months after pollination (Tan, et al., 2014). The composition of MCW differs significantly from that of young coconut. For instance, the predominant sugar shifts from reducing sugar (fructose) to non-

reducing sugar (sucrose). In addition, its relatively high mineral and protein content presents potential for commercialization as a natural rehydration beverage. However, further research on the composition, physicochemical properties and thermal inactivation kinetics of MCW is still required (Tan, et al., 2014).

Coconut water is highly susceptible to microbial storage and oxidative enzymatic deterioration once extracted from the kernel due to its rich nutritional content (Mahayothee, et al., 2016; Tan, et al., 2014). Enzymatic browning during processing and storage requires two key components to initiate the reaction. The primary enzymes involved are polyphenol oxidase (PPO) and peroxidase (POD). Furthermore, naturally occurring phenolic compounds in coconut water act as substrates for PPO activity, a process that can be accelerated by elevated temperatures and metal contamination. According to Garcia, et al. (2007), enzyme activity is more pronounced in young coconut water than in MCW.

Heat treatment has been proven to be the most effective processing method for reducing the microbial load in coconut water and inactivating enzymatic activity at the same time (Tan, et al., 2014). However, excessive heat exposure may lead to undesirable discolouration with yellow, brown or pink colour commonly observed. These colour changes can be attributed either to enzymatic browning or to the Maillard reaction, as coconut water contains both reducing sugars and free amino acids. Therefore, the temperature-time combination applied during heat treatment

must be carefully optimised to minimize such adverse quality changes (Tan, et al., 2014; Sanganamoni, Mahanti and Rao, 2018).

1.2 Problem Statement

Among the available literature, research on processing mature coconut water into ready-to-drink beverages remains relatively limited. In particular, comprehensive information is still lacking regarding the composition of mature coconut water and its response to heat treatment, the impact of enzymatic activity during thermal processing and the discolouration issues associated with both storage and heating. Whether such discolouration is caused by enzymatic or non-enzymatic browning has yet to be clearly defined. Overall, only a few studies are addressing these key factors.

If enzymatic browning causes the discolouration of coconut water during storage, a method to remove the substrate for PPO may be feasible to reduce the discolouration problem. Chitin has been widely applied in wastewater treatment and in the clarification of white wines for the removal of phenolic compounds. Albeit its adsorption efficiency is highly dependent on the surrounding chemical environment. However, the efficiency of chitin in removing phenolic compounds and its compatibility with coconut water have not yet been documented.

1.3 Objectives

The purpose of this study was to achieve these three objectives.

1. To compare the effect of water bath treatment and pressure-cook on polyphenol oxidase and peroxidase activities in mature coconut water.

2. To determine the effect of chitin application on discolouration of water bath-treated and pressure-cooked mature coconut water.

3. To determine the colour changes in treated and untreated mature coconut water as a function of storage time.

CHAPTER 2

LITERATURE REVIEW

2.1 Mature Coconut Water

Mature coconut water (MCW) is the liquid extracted from coconuts that are around 9 months old after pollination. It is regarded as an industrial waste product, as it is often discarded during coconut milk production where only the coconut flesh is used. MCW is generally considered less palatable due to its slightly salty and sourish taste (Tan, et al., 2014; Aziz, et al., 2023). The sugar composition of MCW is primarily sucrose, a non-reducing sugar formed from the conversion of the reducing sugars (fructose and glucose) that dominate in young coconut water. In addition, the total phenolic content, antioxidant properties and protein content increase with maturity. MCW also offers potential health benefits, such as anti-obesity, anti-hypercholesterolemia and antioxidant effects. Moreover, its mineral content increases as the coconut ages, giving the opportunity to market MCW as a rehydration sports drink (Tan, et al., 2014; Aziz, et al., 2023). However, according to Aziz, et al. (2023), in order to market MCW as a ready-to-drink beverages, key challenges include its susceptibility to microbial spoilage and oxidation once opened. As a solution, Tan, et al. (2014) highlighted thermal treatment as an effective method to address these issues by simultaneously reducing microbial load and enzymatic activity.

2.2 Heat Treatment

Heat treatment is one of the most common methods used in food processing for various purposes, such as enhancing palatability, inactivating enzymes, destroying toxins, reducing microbial count and producing a more appealing end product through the development of colour, flavour and aroma, although some of these changes may be undesirable (Inoxmim, 2024). A key factor in obtaining a high quality product is determining the most suitable time-temperature combination and its effect on the food itself.

Since food composition varies widely, understanding the characteristics of a product before subjecting it to heat treatment is crucial, as inappropriate processing may compromise its quality. In the food industry, multiple heat treatment methods are employed, which can generally be categorised into four groups, first, using steam or hot water, second, using air or heated surface, third, using hot oils and fourth, other heat treatment (Aryal, 2023). In this experiment, the two heat treatments selected involved the use of steam or hot water.

2.2.1 Water Bath

Water-bath heating is a conventional method that is relatively low-cost, easy to operate and requires only simple equipment. It has been widely applied in the food industry due to its simplicity and the ability to achieve uniform heat distribution (Naibaho, et al., 2023; Yu, et al., 2024). The heat transfer mechanisms involved in

water-bath heating are conduction and convection. Conduction occurs when the beaker is in direct contact with the hotplate surface, from the beaker wall to the water within and when the hot liquid directly contacts the walls of the universal bottles immersed in the boiling water, while convection takes place within the liquid, where hot liquid rises and cooler liquid sinks. This circulation of liquid molecules enhances heating efficiency and ensure uniform temperature distribution (Traylor, 2023).

2.2.2 Pressure Cook

Using a pressure cooker to prepare food is gaining popularity as it uses saturated steam to heat up food more efficiently and quickly, owing to the increase in the boiling point of water under pressure (Rocca-Poliméni, Flick and Vasseur, 2011). This method excludes oxygen and the reduced cooking time helps preserve vitamins while also improving nutritional and organoleptic qualities. Cooking in a pressure cooker involves three stages. Before the process begins, water, food products and cool air are trapped inside the vessel. In the first stage, heat is applied from the bottom, causing the temperature to rise and the water to heat up, which leads to an increase in pressure due to water vapourization. The valve opens once the internal pressure reaches a certain threshold. In the second stage, the air is displaced by the steam produced and the valve serves the important function of regulating the pressure. A lower air mass fraction leads to water vapour forming droplets on the food's surface. After the designated cooking duration, the power source is shut down and the valve is released. The third stage, the decompression

phase then takes place, during which the pressure drops to atmospheric level. Conduction, convection, evaporation, boiling and condensation are some of the phenomena that occur during pressure cooking (Rocca-Poliméni, Flick and Vasseur, 2011).

Tan, et al. (2014) documented the unfavourable consequences of heat treatment, stating that when young coconut water was heated to 90°C for more than 100 s, it may lose its flavour and develop yellow discolouration. Therefore, heat processing conditions should be optimised based on the response of MCW to heat treatment.

2.3 Chitin

Chitin is a natural biopolymer that can be obtained from diverse sources, such as crustacean shells, fungi, insects and so on, making it readily available. It is the second most abundant polysaccharide after cellulose. However, chitin derived from different sources can exhibit variations in purity, crystallinity, molecular weight and so other physicochemical properties. For instance, insect-derived chitin is considered to be higher quality, involves relatively simpler extraction process and offers greater sustainability, whereas crustacean-derived chitin generally provides higher yields (Izadi, Asadi and Bemani, 2025).

Chitin is a poly-(1→4)-β-linked N-acetyl-D-glucosamine in structure which is similar to the structure of cellulose (**Figure 2.1**). Its deacetylated derivative, chitosan, along with chitin itself has been extensively studied for industrial

applications, particularly in addressing environmental pollution in coastal regions and the seafood-processing industry chitin is highly insoluble and thus chitin from crustaceans and other organisms does not readily degrade (Elieh-Ali-Komi and Hamblin, 2016). Their reactivity is largely attributed to functional groups, hydroxy, acetamido and amino group which enable hydrogen bonds, Van der Waals interactions and ionic interactions. The amino groups, in particular are pH-dependent, where at a low pH, protonation of amino groups enhances electrostatic attraction with negatively charged phenolic compounds.

In the beverage industry, chitin has drawn attraction due to its biocompatibility, biodegradability and low toxicity. Applications include clarification and deacidification of beverages such as wine, beer and fruit juices, antimicrobial activity, edible film formation, preservation of nutritional quality and water purification by removing contaminants (Cosme and Vilela, 2021; Knorr, 2025). However, application of chitin in the food industry is restricted due to its insoluble nature in water, whereas chitosan, being soluble in weakly acidic solutions but not in neutral or alkaline conditions, represents a more suitable alternative for food application (Knorr, 2025).

With regard to phenolic compound removal, chitin has been widely studied in wastewater treatment, where it often achieves higher removal efficiency than chitosan. In contrast, chitosan demonstrates superior adsorption capacity for phenolic compounds in white wine treatment. Despite this, the application of chitin

and chitosan in food industry remains underexplored, highlighting their potential for use in coconut water processing (Spagna, et al., 1996; Pigatto, et al., 2013).

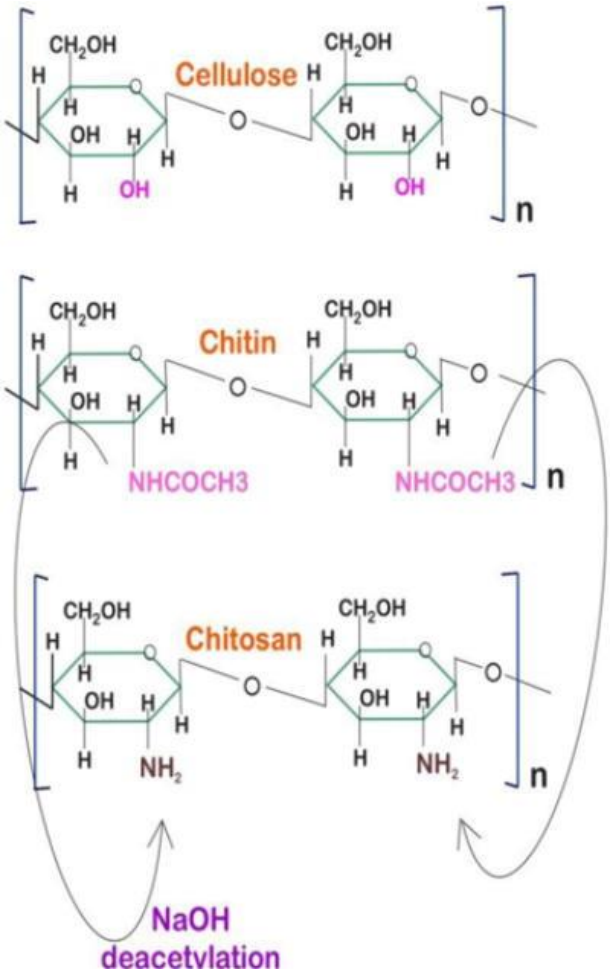

Figure 2.1: Structure comparison between cellulose, chitin and chitosan (Elieh-Ali-Komi and Hamblin, 2016).

2.4 Factors Lead to Discolouration of Fruit Juice or Beverages

Discolouration refers to an undesirable change in colour, which negatively affects the visual appeal of beverages. For instance, discoloured beverages are normally seen as being of lower quality because colour is one of the most significant

variables determining consumer acceptance. Discolouration is commonly observed in fruit juices during storage or heat treatment and it can result from either non-enzymatic browning or enzymatic browning. Non-enzymatic browning is mainly attributed to the Maillard reaction, ascorbic acid degradation and caramelization, whereas enzymatic browning depends on the presence of enzymes, phenolic compounds and oxygen (Bharate and Bharate, 2012). Enzymes is a biological catalyst, that is made of protein to speed up the chemical reaction without being consumed. Classification of enzymes are made based on their working mechanism. For instance, oxidoreductases, lyases, ligases, hydrolases and isomerases are the six major classes. Polyphenol oxidase (PPO) and peroxidase (POD) falls in the class of oxidoreductase (De Oliveira, Santos and Buffon, 2021).

The main discolouration problem reported in coconut water is pink discolouration. According to Kanjanapongkul and Baibua (2021), pink discolouration in coconut water is mainly attributed to PPO activity. This phenomenon is observed particularly in water bath-treated coconut water and in young coconut waters, which exhibit higher enzyme activity compared to mature coconut (Tongdonyod, et al., 2023; Garcia, et al., 2007).

2.5 Enzymatic Browning

According to Rajashri, et al. (2019), the browning in coconut water was mainly caused by polyphenol oxidase (PPO) and peroxidase (POD). The sensory and nutritional value of coconut water declines when enzymatic browning takes place.

PPO employs a mechanism where phenolic compounds serve as the substrate and undergo a series of polymerization to produce a brown or black pigment. In contrast, POD enhances the phenolic oxidation reaction.

2.5.1 Polyphenol Oxidase

Polyphenol oxidase (PPO) is an oxidative enzyme, also referred to as catechol oxidase and tyrosinase. It is the key enzyme responsible for the browning effect observed in many fruits and vegetables (Qu, et al., 2025). PPO is a copper-containing enzyme with two copper ions coordinated by histidine residues at the catalytic site. The active site is located within four α-helix bundles, which form the core of the enzyme's structure. These central helices contain the catalytic binuclear copper centre, where three histidine residues from the surrounding α-helices coordinate each of the two copper ions (Qu, et al., 2025; Murtaza, et al., 2018).

PPO functions by oxidising polyphenols and breakdown of polyphenols and their derivatives. It is released upon cell damage, allowing phenolic compounds to come into contact with PPO, where reactions occur in the presence of oxygen (Toro-Uribe, et al., 2020). In the process, oxygen serves as the electron acceptor in the oxidation mechanism of PPO. The reaction proceeds in two steps. First, hydroxylation of monophenols is catalyzed to form *o*-diphenols. Then, the *o*-diphenols are oxidised to yield o-quinones. These highly reactive *o*-quinones subsequently undergo non-enzymatic polymerization, producing melanin-like compounds that impart a brown or black colour (**Figure 2.2**). The PPO activity depends on several factors,

including the concentration of phenolic compounds, oxygen availability, pH, temperature and so on (Toro-Uribe, et al., 2020). In the case of coconut water, pink discolouration has been reported during storage, attributed to the combined action of PPO and peroxidase (POD) (Syahputri, Santoso and Supriyanto, 2021).

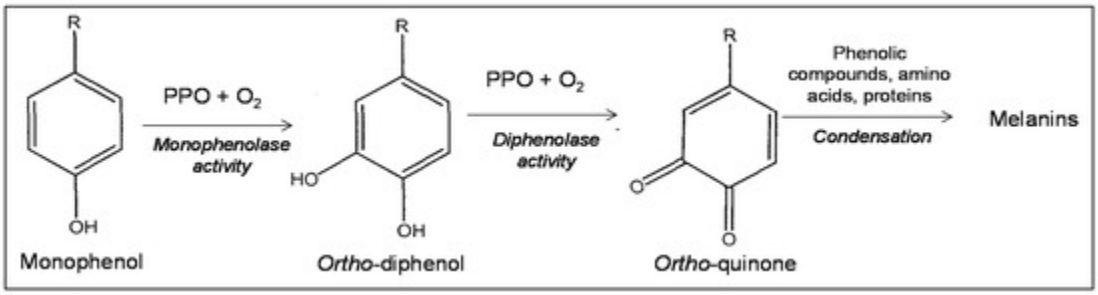

Figure 2.2: Schematic illustration of PPO-catalyzed enzymatic browning reaction (Taranto, et al., 2017).

2.5.2 Peroxidase

Peroxidase (POD) is an oxidoreductase that facilitates the oxidation of various substrates by utilising hydrogen peroxide as the oxidising agent. Substrates such as phenolic compounds, cytochrome c, ascorbic acid and various other molecules can act as hydrogen donors in these reactions. POD are generally classified into two main groups, heme and non-heme peroxidases, depending on the presence of heme group and serve different functions (Chukwudi, Onyetugo and Amarachi, 2021). POD are ubiquitous and can be found in microorganisms, plants and animals. The greatest diversity of peroxidases can be found in plants, as evidenced by the more than 40 different isoforms of horseradish peroxidase (HRP). In addition to classical peroxidase reactions, POD also involves in aerobic oxidations, hydroxylation of aromatic compounds, halogenation and so on with hydrogen peroxide as a crucial reactant. Their widespread presence and remarkable catalytic diversity highlight

their importance, although their roles remain underexplored (Chukwudi, Onyetugo and Amarachi, 2021; De Oliveira, Santos and Buffon, 2021).

2.5.3 Total Phenolic Content

Polyphenolic compounds are a diverse group of antioxidants known for numerous health benefits, including cardiovascular protection, antioxidant activity, anti-inflammatory effects and so on (Toro-Uribe, et al., 2020). They encompass a wide range of subclasses, such as lignans, phenolic acids, flavonoids, stilbenes, coumarins and other low-molecular-weight compounds. In coconut water, salicylic acid, catechins and epicatechins are the major phenolic compounds responsible for its antioxidant potential. Other phenolics including coumaric acid, caffeic acid and so on are also present in a small amount. These compounds act as substrates for enzymatic browning reactions (Syahputri, Santoso and Supriyanto, 2021).

The Folin-Ciocalteu (F-C) assay was employed in this experiment to quantify phenolic compounds in mature coconut water (MCW) samples. It was initially employed in wine research, but it has since developed into the standard method for detecting and quantifying phenolic compounds in a variety of dietary and biological samples (Pérez, Dominguez-López and Lamuela-Raventós, 2023). The F-C assay is widely used because it is cost-effective, reproducible and compatible with standard laboratory equipment, making it easy to incorporate into different laboratory settings. It is very sensitive and can be effectively used to analyze

complex phenolic compounds in fruits, vegetables and other foods. The F-C reagent, which is yellow due to phosphomolybdic acid and phosphotungstic acid (Pérez, Dominguez-López and Lamuela-Raventós, 2023).

The F-C assay operates on a single electron transfer (SET) mechanism, where the F–C reagent acts as the oxidant and phenolic compounds serve as reducing agents (**Figure 2.3**). In alkaline conditions, phenolic compounds reduce the phosphomolybdic acid and phosphotungstic acid, producing a blue chromophore that can be detected at 760-765 nm wavelength. The intensity of the blue colour is directly proportional to the reducing capacity of the phenolics, with results typically expressed as gallic acid equivalents (GAE) (Pérez, Dominguez-López and Lamuela-Raventós, 2023).

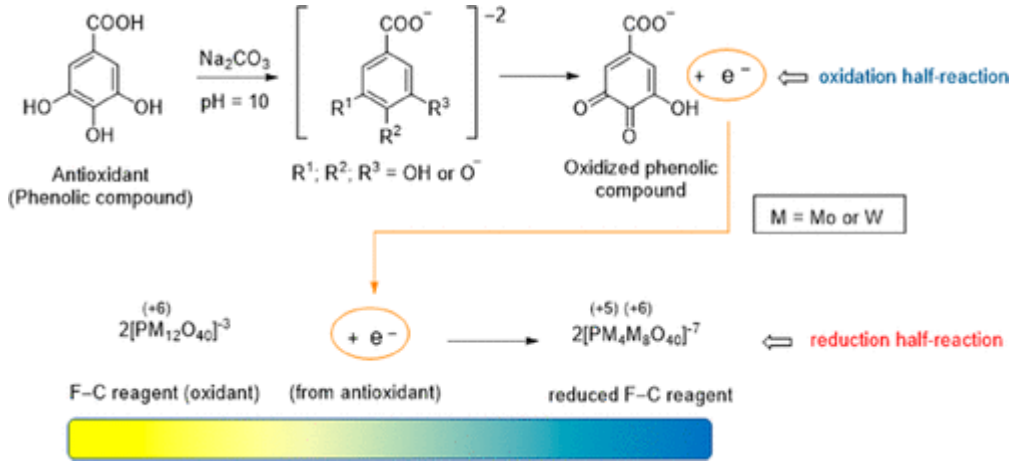

Figure 2.3: The redox reaction involved in the Folin-Ciocalteu assay (Pérez, Dominguez-López and Lamuela-Raventós, 2023).

Despite its widespread use, the F-C assay lacks specificity for phenolic compounds, since non-phenolic substances may also react with the reagent, potentially leading

to overestimation. Nevertheless, it remains a reliable and widely accepted method for total phenolic content (TPC) determination (Pérez, Dominguez-López and Lamuela-Raventós, 2023).

2.6 Colour

Colour is an important attribute that can influence consumers perception on the fruit juices in terms of quality, nutritional value and freshness. One of the most used colour spaces for measuring object colour is the CIELAB system. It is a standard tool in the colour control and management sectors as it is standardised and it accurately reflect how human perceived the colour. It has gained international recognition and is now the method of choice for scientific studies on the colour of fruit juices (Kardas, et al., 2023).

Three parameters, L* for lightness, a* for green (-) to red (+) colour and b* for blue (-) to yellow (+) colour are the fundamentals of the CIELAB system. Perception of brightness, hue and intensity can then be measured accurately from these three parameters. It is crucial in sensory evaluation of fruit juices, colour changes monitoring in products that are prone to natural oxidation or enzymatic browning (Kardas, et al., 2023).

Browning index (BI) is a quantitative measure used to indicate the degree of browning. It is applied in various studies to assess the extent of enzymatic or non-

enzymatic browning as well as colour changes during storage or heat processing. BI is calculated using all three CIELAB parameters, L*, a* and b*. A higher BI value indicates greater browning (Cefola, 2012).

2.7 Microbial Quality in Relation to Industrial Sterility and Shelf Life

2.7.1 Total Plate Count

Total plate count is a conventional microbiological method used to estimate microbial load and assessing food quality. The sample is first serially diluted before being spread on a solid agar medium for colony- forming unit (CFU) enumeration. Each visible colony is generally assumed to originate from a single cell, however, this does not account for aggregated colonies (Di Caprio, 2020). A major limitation of total plate count is that it excludes inactive, viable but non-culturable (VBNC) and nonculturable bacteria, leading to underestimation (Alsanius and Wohanka, 2019). Furthermore, this method only provides information on the total microbial load instead of identifying specific microbial types (Brackett, Shewfelt and Prussia, 1993).

2.7.2 Plate Count Agar

Plate count agar (PCA), also known as Tryptone Glucose Yeast Agar or Casein-Peptone Dextrose Yeast Agar is a non-selective medium commonly used to enumerate viable aerobic bacteria in a sample. PCA is composed of enzymatic digest of casein (tryptone), yeast extract, glucose and agar. The enzymatic digest of

casein primarily supports microbial proliferation, yeast extracts provide B-complex vitamins, glucose serves as the carbon source for bacterial growth and agar functions as the solidifying agent (Aryal, 2022).

2.7.3 Spread Plate Method

The spread plate technique is a widely used microbiological method for isolating and quantifying viable microorganisms in a liquid sample. It is simple and relatively easy to perform. A pre-diluted sample that is spread evenly over the surface of solidified agar using a sterile spreader. The plate is then incubated and colonies are counted to determine CFU/mL. A successful spread plate produces a uniform distribution of colonies, with only counts within the range of 30-300 CFU/mL considered acceptable. Colonies counting is easier with the spread plate method than with the pour plate method, as colonies are distributed on the surface rather than being embedded in the agar. However, this method may underestimate microbial numbers as only viable, aerobic bacteria grow. In addition to enumeration, the technique is also employed in enrichment, selection and screening studies (Dahal, 2022; Sanders, 2012).

According to Adolf, Edna and Rebecca (2012), *Escherichia coli* and *Klebsiella pneumoniae* are potential contaminants in coconut water due to its nutrient-rich composition. Both *E. coli* and *K. pneumoniae* are facultative anaerobes, capable of surviving under aerobic and anaerobic conditions (Chen, Nicolau and Kuti, 2019).

Therefore, the spread plate method was employed in the experiment for its simplicity and ease of preparation.

CHAPTER 3

METHODOLOGY

3.1 Experimental Flowchart

Figure 3.1 illustrates the overall flow of the experiment, including the treatments applied and the analyses carried out. A total of 5 treatments were applied in the experiment. For all treatments, total phenolic content (TPC), polyphenol oxidase (PPO) activity, peroxidase (POD) activity and colour were determined on Days 0, 4, 8 and 12. Total plate count was performed only on Day 12 to determine the sterility of the treated samples.

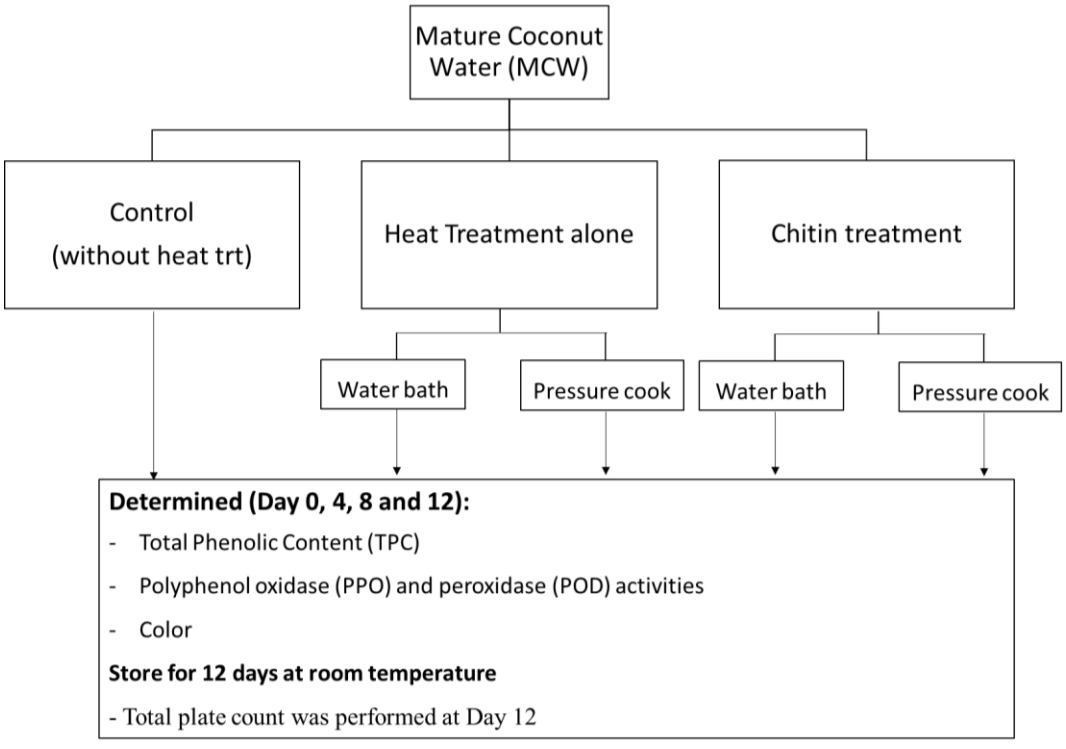

Figure 3.1: The overview of the experiment.

3.2 Materials

3.2.1 Sample Materials

Two mature coconuts were obtained from a wet market in Kampar, Perak. The coconuts were freshly purchased prior to the experiment and sourced from the same vendor as those used in preliminary study in order to ensure consistency.

3.2.2 Chemicals

The sources of chemicals used in the project are listed in **Table 3.1**.

Table 3.1: Sources of chemicals used for reagents preparation and analysis.

Chemicals	Sources	Used in Method
Chitin	R&M Chemicals	3.4 Pre-treatment with Chitin
Monosodium phosphate	HmbG Chemicals	3.6.1 Reagent Preparation
Disodium hydrogen phosphate	Bio Basic Canada	3.6.1 Reagent Preparation
0.1N Hydrochloric acid	QReC^{TM}	3.6.1 Reagent Preparation
Sodium hydroxide	Chemiz	3.6.1 Reagent Preparation
Pyrocatechol	R&M Chemical	3.6.1 Reagent Preparation
Guaiacol	Merck	3.6.1 Reagent Preparation
Hydrogen peroxide	Friendemann Schmidt	3.6.1 Reagent Preparation
Gallic acid	R&M Chemical	3.7.2 Determination of Total Phenolic Content
Sodium carbonate	Chemiz	3.7.1 Reagent Preparation
Folin-Ciocalteu's phenol reagent	Merck	3.7.2 Determination of Total Phenolic Content
Sodium chloride	Chem Soln	3.9.1 Preparation of Diluent

Table 3.1 (continued): Sources of chemicals used for reagents preparation and analysis.

Plate Count Agar	Merck	3.9.2 Preparation of Plate Count Agar

3.2.3 Equipment

All the sources of equipment used for analysis are listed in **Table 3.2**.

Table 3.2: Sources of equipment used for analysis.

Equipment	Brand, Model, Country	Used in Method
Incubator Shaker	INFROS HT / Ecotron	3.4 Pre-treatment with Chitin
Water bath	Memmert, WNB, Germany	3.6.2 Determination of Polyphenol Oxidase Activity
		3.6.3 Determination of Peroxidase Activity
UV-Vis Spectrophotometer	Thermo Scientific, Genesys 10S UV-Vis, United States	3.6.2 Determination of Polyphenol Oxidase Activity
		3.6.3 Determination of Peroxidase Activity
		3.7.2 Determination of Total Phenolic Content
Incubator	Memmert, ICO105, Germany	3.9.3 Determination of Total Plate Count
Hotplate	IKA, RCT basic, Malaysia	3.5.1 Water Bath
		3.6.1 Reagent Preparation
		3.7.1 Reagent Preparation
		3.9.1 Preparation of Diluent
		3.9.2 Preparation for Plate Count Agar
Weighing balance	Mettler Toledo, ME1002, Switzerland	3.4 Pre-treatment with Chitin
		3.6.1 Reagent Preparation

Table 3.2 (continued): Sources of equipment used for analysis.

		3.7.1 Reagent Preparation 3.9.1 Preparation of Diluent 3.9.2 Preparation of Plate Count Agar
Colorimeter	Konica Minolta, CM-600d, Japan	3.8 Colour Measurement

3.3 Sample Preparation

The mature coconuts were first placed in a laminar flow hood and their surfaces were sterilized using UV illumination. After 15 min, each coconut was drilled with two holes to extract the water which was then filtered through a sterile mesh cloth into a conical flask. The coconut water was divided into two sets, with one set prepared for pre-treatment with chitin and the remainder was used for heat treatment alone.

3.4 Pre-treatment with Chitin

The chitin treatment was carried out according to Pigatto, et al. (2013) and Dursun and Kalayci (2005) with slight modifications. A total of 4 conical flasks were each filled with 100 mL of filtered MCW. A chitin concentration of 4 g/L was used, with 0.4 g added to each conical flask. The flasks were incubated in an incubator shaker at 150 rpm and 25°C for 4 h. After incubation, the MCW was filtered through mesh cloth and the filtrates (MCW) were combined into a single conical flask. Then, 15 mL of the treated MCW was then aliquoted into a series of universal bottles, with each containing 15 mL. A total of 24 bottles were prepared, with 12 bottles for CW

and 12 bottles for CP. The corresponding thermal treatment was then applied to these samples.

3.5 Heat Treatment

There were five treatments in total, which was control, water bath treatment (W), pressure-cook treatment (P), chitin with water bath treatment (CW) and chitin with pressure-cook treatment (CP). Four sets of samples (exclude the control without any treatment) were undergone either heat treatment alone or a combination of chitin plus heat treatment in triplicate for analysis on Days 0, 4, 8 and 12. On each scheduled examination day, a fresh set of 15 samples was retrieved for the analysis of enzyme activities, total phenolic content (TPC) and colour determination. Analyses were carried out at four-day intervals (refer to **Table 3.3**).

For samples undergoing heat treatment alone, the MCW was aliquoted into a series of universal bottles, with each containing 15 mL. A total of 36 bottles were prepared with 12 bottles for control, 12 bottles for W and 12 bottles for P (refer to **Table 3.3**). On the other hand, samples subjected to pre-treatment with chitin prior to heat treatment were prepared as described in **Section 3.4**.

Table 3.3: Number of samples prepared for control and four treatments (W, P, CW and CP) for analysis on Day 0, 4, 8 and 12.

	Number of samples (n)					
Day	**Control**	**Water Bath (W)**	**Pressure Cook (P)**	**Chitin + Water Bath (CW)**	**Chitin + Pressure Cook (CP)**	**Total**
0	3	3	3	3	3	**15**
4	3	3	3	3	3	**15**
8	3	3	3	3	3	**15**
12	3	3	3	3	3	**15**
Total	12	12	12	12	12	

Two heat treatments were selected to reduce the microbial count in the MCW samples. A total of 24 bottles were performed for water bath treatment, with 12 bottles for W and 12 bottles for CW and 24 bottles for pressure-cook treatment with 12 bottles for P and 12 bottles for CP (refer to **Table 3.3**).

3.5.1 Water Bath

First, 400 mL of distilled water was boiled in a beaker on a hotplate. Once boiling, 3 bottles containing pre-filled MCW were immersed in the boiling water and a 20-min heating timer was started. The boiling condition was maintained throughout the 20 min. After heating, the bottles were immediately inserted into an ice bath to cool and to prevent further reactions. The process was carried out in a total of 8 rounds, comprising 4 rounds for W samples and 4 rounds for CW samples, with each round consisting of 3 bottles. The samples were then stored at room temperature for further analysis.

3.5.2 Pressure Cook

A pressure cooker (Midea, model MY-12LS605A) was used in the experiment. The voltage, pressure and power of the pressure cooker were 240 V-50 Hz, 70 kPa and 1000 W respectively. The pressure cooker was filled with approximately 300 mL of water and a provided steamer rack was placed inside. A total of 12 bottles were arranged on the rack. The soup program was selected to initiate the process. Approximately 5 min were required for the water to be heated and for the pressure to build up in the vessel. A 20-min heating period was started when the pressure indicator raised. After heating, the bottles were immediately transferred into an ice bath to cool and to prevent further reactions. One round was conducted with a total of 12 bottles. Another round was performed for samples of CP. The samples were then stored at room temperature for further analysis.

3.6 Enzyme Activities

3.6.1 Reagent Preparation

All reagents were freshly prepared prior to analysis.

To prepare 0.2 M sodium phosphate buffer, 1.2 g of monosodium phosphate and 1.42 g of disodium hydrogen phosphate were dissolved in 50 mL distilled water. The stock solution was then divided equally into two portions of 25 mL each. The pH of the portions was adjusted to 5.5 and 6.0 respectively using 0.1 M hydrochloric acid and 0.1 M sodium hydroxide. Each portion was then topped up to 50 mL with distilled water after the desired pH had been reached.

To prepare 0.2 M pyrocatechol, 0.22 g of pyrocatechol was dissolved in 7 mL of distilled water in a beaker. The solution was heated gently on a hotplate to aid dissolution. Once the pyrocatechol solid had fully dissolved, the solution was then transferred to a 10 mL-volumetric flask and topped up to the calibration mark with distilled water.

3.6.2 Determination of Polyphenol Oxidase Activity

The determination of PPO activity was carried out according to Tan, et al. (2014) with slight modifications. A new set of samples (15 bottles) was used for analysis at each interval (Days 0, 4, 8 and 12), with three replicates for each treatment. The PPO activity in the MCW treatments were determined using pyrocatechol as the substrate. First, a solution mix containing 22 mL of 0.2 M phosphate buffer (pH 6.0) and 6 mL of 0.2 M pyrocatechol solution was prepared in a centrifuge tube. The mixture was incubated in a 25℃ water bath for 5 min to stabilise its temperature. After incubation, a volume of 1.75 mL of the solution mix was transferred into a cuvette, followed by the addition of 0.025 mL of the sample. The cuvette was covered with parafilm and inversed several times for thorough mixing. Absorbance was then measured at 425 nm every 30 s for 3 min. A blank was prepared by mixing 1.75 mL of the incubated solution with 0.025 mL of distilled water. The steps above were repeated with another 14 samples. One unit of enzyme activity was defined as the amount of enzyme that caused increase in absorbance at a rate of 0.001 unit per min.

The enzyme activity was calculated based on the equation below:

$$Enzyme\ activity\ (U/ml) = \frac{absorbance}{0.001 \times 0.025\ ml}$$

3.6.3 Determination of Peroxidase Activity

The determination of POD activity was carried out according to Tan, et al. (2014) with slight modifications. A new set of samples (15 bottles) was used for analysis at each interval (Days 0, 4, 8 and 12), with triplicates for each treatment. The POD activity in the MCW treatments were determined using guaiacol as the substrate. First, a solution mix containing 22.4 mL of 0.2 M phosphate buffer (pH 5.5), 4.8 mL of 0.05% guaiacol and 1.6 mL of 0.1% hydrogen peroxide was dispensed into a centrifuge tube. The mixture was incubated in a 35℃ water bath for 5 min to stabilise its temperature. After incubation, a volume of 1.8 mL of the solution mix was transferred into a cuvette, followed by the addition of 0.020 mL of the sample. The cuvette was covered with parafilm and inversed several times for thorough mixing. Absorbance was then measured at 470 nm every 30 s for 3 min. A blank was prepared by mixing 1.8 mL of the incubated solution with 0.020 mL of distilled water. The steps above were repeated with another 14 samples. One unit of enzyme activity was defined as the amount of enzyme that caused increase in absorbance at a rate of 0.001 unit per min.

The enzyme activity was calculated based on the equation below:

$$Enzyme\ activity\ (U/ml) = \frac{absorbance}{0.001 \times 0.020\ ml}$$

3.7 Total Phenolic Content

3.7.1 Reagent Preparation

To prepare 20% sodium carbonate solution, 10 g of sodium carbonate was dissolved in 20 mL of distilled water in a beaker. The solution was heated gently on a hotplate to aid dissolution. Once the sodium carbonate solid had fully dissolved, the solution was then transferred to a 50 mL volumetric flask and topped up to the calibration mark with distilled water.

3.7.2 Determination of Total Phenolic Content

The determination of TPC was carried out according to Tan, et al. (2014) and Tongdonyod, et al. (2023) using Folin-Ciocalteu's method with slight modifications. The total phenolic content in the MCW samples were determined by Folin-Ciocalteu's method. A new set of samples (15 bottles) was used for analysis at each interval (Days 0, 4, 8 and 12), with triplicates for each treatment. First, a volume of 0.2 mL samples and different concentration of gallic acid standard solution (refer to **Table 3.4**) were added into a total of 15 microcentrifuge tubes respectively. Then, 0.1 mL of Folin-Ciocalteu's phenol reagent and 0.8 mL of distilled water were added into all microcentrifuge tubes. The mixtures were then incubated at room temperature for 3 min and light was avoided by keeping in drawer. After incubation,

0.3 mL of 20% sodium carbonate was added into all microcentrifuge tubes followed by an incubation time of 120 min at room temperature and light was avoided by keeping in drawer. A blank was prepared by following the same steps, with 0.2 mL of the sample substituted with distilled water. Absorbance was then measured at 765 nm. Lastly, gallic acid standard curve was plotted and the concentration of total phenolic content in the samples were calculated. The concentration of TPC was expressed as gallic acid equivalents (GAE) using units of mg/L (mg GAE/L).

Table 3.4: The concentration of standard gallic acid solution used to plot standard curve.

Volume of gallic acid (µl)	Volume of distilled water (µl)	Concentration of gallic acid (mg/mL)
0.00 (blank)	200.00	0
20.00	180.00	0.02
40.00	160.00	0.04
60.00	140.00	0.06
80.00	120.00	0.08

Note: Concentration of gallic acid stock solution was 0.2 mg/mL

3.8 Colour Measurement

The colour of MCW samples were determined using colourimeter with SCE mode. A new set of samples (15 bottles) was used for analysis at each interval (Days 0, 4, 8 and 12), with triplicates for each treatment. The measurements were based on the CIE-Lab colour space parameters. L* represents lightness, with values ranging from 0 (black) to 100 (white). The colour redness is indicated by a positive a* value

and green colour is indicated by a negative a* value. Yellow colour is indicated by a positive b* value, and negative b* value indicates blue colour (Ding and Ling, 2014). First, zero calibration was performed based on the baseline reflectance measurement from the surface and white calibration was done with the calibration cap provided to ensure accuracy. Liquid samples were then placed in a small petri dish for measurement and data were collected. The measurement was repeated for a total of 15 samples at each interval.

3.8.1 Calculation for Browning Index

The browning index was calculated according to Ding and Ling (2014) using the following equation:

$$X = \frac{a^* + 1.75\ L^*}{5.645\ L^* + a^* - 3.012\ b^*}$$

$$Browning\ Index\ (BI) = \frac{100\ (X - 0.31)}{0.17}$$

3.9 Total Plate Count

3.9.1 Preparation of Diluent

A 0.85% NaCl solution was used as the diluent. A total of 50 mL was prepared by dissolving 0.425 g of sodium chloride in 20 mL of distilled water in a beaker. The solution was heated on a hotplate to aid dissolution. Once the sodium chloride had fully dissolved, the solution was transferred into a 50 mL volumetric flask and

diluted to the calibration mark with distilled water. The diluent was then transferred into a 100 mL Schott bottle and autoclaved.

3.9.2 Preparation for Plate Count Agar

Total plate count was performed on Day 12 to assess the sterility of the MCW samples and to ensure their safety for consumption during storage at room temperature for up to 12 days. The microbial test was conducted using the spread plate method according to Tongdonyod, et al. (2023) with slight modifications.

First of all, plate count agar (PCA) was prepared by mixing 6.75 g of PCA powder with 300 mL of distilled water in a 500 mL Schott bottle. The mixture was heated slightly on a hotplate to aid dissolution. Once the PCA powder had completely dissolved, the bottle was capped and autoclaved. The procedure was repeated to prepare an additional 300 mL of PCA. The autoclaved molten agar was then kept in an oven at 70℃ to maintain in its molten state.

A total of 30 Petri plates were prepared and sterilized in the laminar flow hood. After UV illumination, PCA agar was poured into the sterile Petri plates. Each plate was filled with approximately 15 mL of molten agar and left to cool and solidify. The plates were then incubated at 37℃ overnight to ensure no contamination was present in the incubator. Preparation of PCA agar was carried out one day prior to the spread plate microbial test.

3.9.3 Determination of Total Plate Count

The spread plate method was carried out using serial dilutions of samples from WB, P, CW and CP retrieved from Set 4 on Day 12. Triplicates were performed for each treatment. First, 10 µL of the sample (10^0 dilution) was transferred into a sterile microcentrifuge tube containing 990 µL of 0.85% NaCl diluent to obtain the 10^{-2} dilution. Next, 100 µL of the 10^{-2} dilution was transferred into another sterile microcentrifuge tube followed by the addition of 900 µL of diluent to obtain 10^{-3} dilution. After serial dilution, 80 µL of the 10^{-2} dilution was transferred onto a sterile PCA agar and spread using a sterile glass spreader. The plate was then sealed with parafilm and incubated at 37°C in an inverted position for 24 hours. A total of 6 plates were done for each treatment, with 2 plates per replicate (10^{-2} and 10^{-3} dilutions), giving a total of 24 plates. After 24 hours of incubation, the results were observed and recorded. Plates containing 30-300 colonies were used to determine the total colony forming unit (CFU/mL).

3.10 Statistical Analysis

All analyses were performed in triplicate for every treatment (n=3). The results were expressed as mean ± standard deviation. Statistical analyses were performed using IBM SPSS Statistics software (version 27), with the level of significance set at $p < 0.05$. A two-way analysis of variance (ANOVA) was conducted to evaluate the effects of treatment and storage time. The dataset was first split by treatment to determine the significance of storage time within each treatment group. Then, the dataset was split by storage time to determine significant difference between

treatments at particular storage days. When the results showed significant differences, Tukey's HSD test was employed to determine which treatments and storage days differed significantly. The two-way ANOVA was carried out on the analyses of polyphenol oxidase (PPO) activity, total phenolic content (TPC) and browning index (BI).

CHAPTER 4

RESULTS

4.1 Changes in Polyphenol Oxidase Activity

Table 4.1 and **Figure 4.1** show the PPO activity and its changes throughout 12 days storage. The control exhibited a significantly highest PPO activity among all samples on Day 0 ($p < 0.05$). It then decreased sharply from Day 0 to Day 4 ($p < 0.05$), slightly increased on Day 8 ($p < 0.05$), and then declined again by Day 12 ($p < 0.05$). The control remained the highest in PPO activity throughout the 12-days storage ($p < 0.05$).

To compare the effect of heat treatments, PPO activity in the water bath-treated sample (W) dropped from Day 0 to Day 4 (154.67 to 40.00 U/mL) and then remained stable up to Day 12. On the other hand, PPO activity in pressure-cooked sample (P) declined to undetectable levels from Day 0 to 4 and remained absent on Days 8 and 12. W and P were significantly different on Days 0, 4, 8 and 12, with P exhibiting lower PPO activity compared to W ($p < 0.05$). Since lower PPO activity was observed in P, no significant browning ($p > 0.05$) occurred in P from Day 0 to Day 12, whereas W exhibited significant browning ($p< 0.05$) as shown in **Table 4.3**. Thus, PPO activity may serve as an indicator for predicting the occurrence of enzymatic browning.

To compare the effect of chitin treatment, both pressure-cooked samples, without or with chitin treatment (P and CP), showed the lowest PPO activity on Day 0, with no significant difference between them (58.00 U/mL and 40.00 U/mL respectively; $p > 0.05$). No detectable PPO activity in both samples on Days 4, 8 and 12. For the water bath-treated samples, PPO activity was similar between W (154.67 U/mL) and CW (128.00 U/mL) on Day 0, but CW showed significantly higher activity on Day 4 while lower activity on Day 12. At Day 8, both samples maintained comparable PPO activity.

Table 4.1: PPO activity of control and treated samples (WB, P, CW, CP) during 12 days of storage.

Treatments	PPO activity			
	Day 0	**Day 4**	**Day 8**	**Day 12**
Control	520.00 ± 0.00^{cC}	252.00 ± 16.97^{aD}	324.00 ± 28.28^{bC}	230.67 ± 12.22^{aD}
W	154.67 ± 28.10^{bB}	40.00 ± 5.66^{aB}	50.00 ± 2.83^{aB}	64.00 ± 0.00^{aC}
P	58.00 ± 8.49^{bA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}
CW	128.00 ± 5.66^{cB}	96.00 ± 11.31^{bC}	52.00 ± 5.66^{aB}	40.00 ± 0.00^{aB}
CP	40.00 ± 5.66^{bA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}

Values are expressed as mean ± standard deviation (n=3). Means with different lowercase superscripts indicate significant differences between days (within row) at $p < 0.05$. Means with different uppercase superscripts indicate significant differences between treatments (within column) at $p < 0.05$.

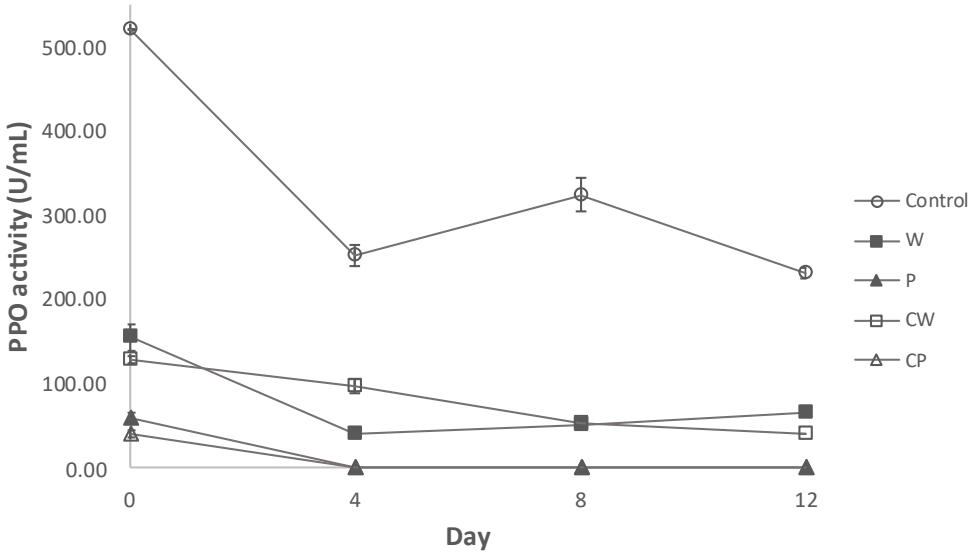

Figure 4.1: Changes in PPO activities of control and treated samples (W, P, CW, CP) during 12 days storage.

4.2 Changes in Peroxidase Activity

Throughout the storage period (Days 0, 4, 8 and 12), no POD activities were detected in any sample.

4.3 Changes in Total Phenolic Content

Table 4.2 and **Figure 4.3** show the TPC and its changes throughout 12 days storage. The TPC of control and samples treated with different treatments were calculated based on the equation obtained from the standard curve of gallic acid shown in **Figure 4.2**. A linear equation of **y= 13.715x + 0.0232** with regression coefficient **R^2= 0.9975** were obtained.

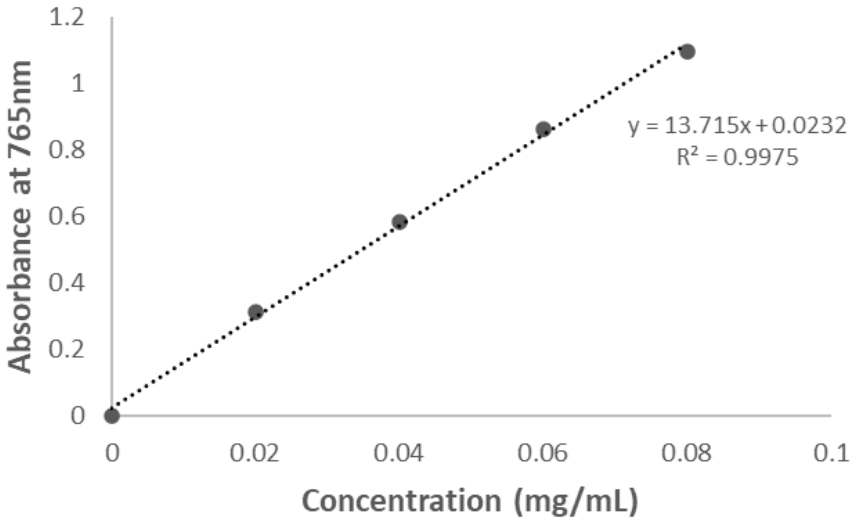

Figure 4.2: Standard curve of gallic acid.

As shown in **Table 4.2**, TPC of control increased significantly from Day 0 (53.50 mg GAE/L) to Day 8 (73.90 mg GAE/L; $p < 0.05$) before declining by Day 12 (66.56 mg GAE/L; $p < 0.05$). Both water bath-treated (W) and pressure-cooked (P) samples showed no significant difference at all time intervals ($p > 0.05$). For W, a significant decrease ($p < 0.05$) in TPC was observed from Day 4 (44.46 mg GAE/L) to Day 8 (39.94 mg GAE/L) and maintained stable at Day 12. On the other hand, P experienced a significant decrease ($p < 0.05$) from Day 0 (47.60 mg GAE/L) to Day 8 (40.26 mg GAE/L), followed by an increase to 45.46 mg GAE/L by Day 12 ($p < 0.05$).

To compare the effect of chitin treatment, CW showed similar TPC to W on Day 0 and Day 4. By Day 8, TPC in W decreased, resulting in a significantly lower value compared to CW ($p < 0.05$). By Day 12, CW exhibited higher TPC than W ($p < 0.05$), as TPC in CW increased from 46.58 mg GAE/L (Day 8) to 56.53 mg GAE/L

(Day 12). On the other hand, CP consistently exhibited higher TPC than P across all examined time points ($p < 0.05$).

Table 4.2: TPC of control and treated samples (WB, P, CW, CP) during 12 days of storage.

	Total Phenolic Content (mg GAE/L)			
	Day 0	**Day 4**	**Day 8**	**Day 12**
Control	53.50 ± 0.82^{aB}	71.49 ± 1.68^{bcC}	73.90 ± 2.35^{cC}	66.56 ± 2.71^{bC}
W	42.49 ± 1.55^{abA}	44.46 ± 1.93^{bA}	39.94 ± 0.41^{aA}	41.33 ± 0.15^{abA}
P	47.60 ± 1.24^{bAB}	48.47 ± 2.48^{bA}	40.26 ± 0.15^{aA}	45.46 ± 0.97^{bA}
CW	46.92 ± 3.33^{aA}	45.51 ± 2.49^{aA}	46.58 ± 0.10^{aB}	56.53 ± 2.84^{bB}
CP	61.50 ± 0.36^{cC}	54.21 ± 1.81^{bB}	47.82 ± 0.21^{aB}	54.31 ± 2.56^{bB}

Values are expressed as mean ± standard deviation (n=3). Means with different lowercase superscripts indicate significant differences between days (within row) at $p < 0.05$. Means with different uppercase superscripts indicate significant differences between treatments (within column) at $p < 0.05$.

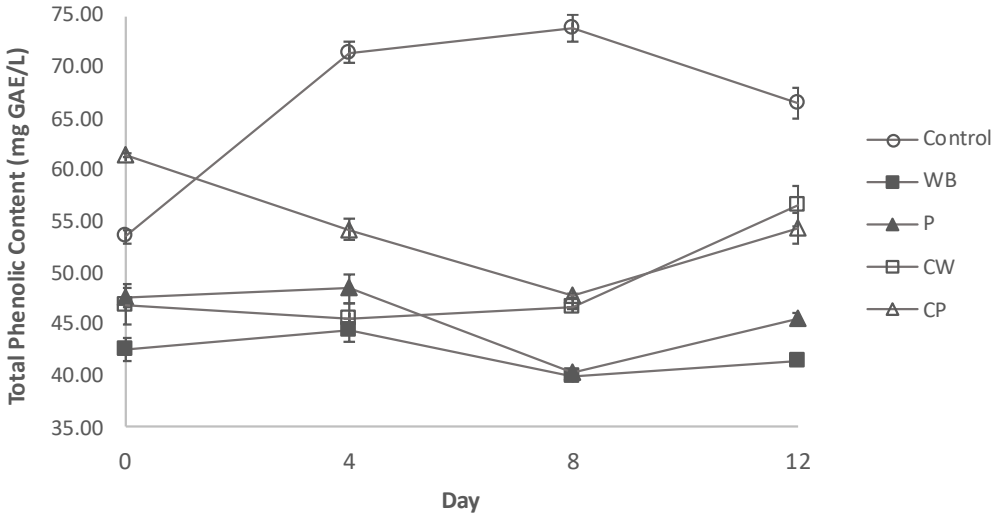

Figure 4.3: Changes in TPC of control and treated samples (W, P, CW, CP) during 12 days storage.

4.4 Changes in Browning Index

Table 4.3 and **Figure 4.4** show the browning index and its changes throughout 12 days storage. The effect of treatments on the browning index during 12 days of storage is shown in **Figure 4.4**. The control exhibited a significant increase from Day 0 to Day 8 (-4.65 to -2.01, $p < 0.05$), but no significant difference from Day 8 to Day 12. For both W and CW, no significant differences were observed from Day 0 to Day 8 ($p > 0.05$), whereas a significant increase was observed from Day 8 to Day 12 (-4.62 to -3.22 and -4.16 to -2.89 respectively; $p < 0.05$). BI remained consistent over 12 day-storage for both P and CP ($p > 0.05$).

On Day 0, no significant browning was observed in any of the treatments (W, P, CW and CP) compared to the Control ($p > 0.05$). The two heat treatments, W and P showed similar BI values ($p > 0.05$). Likewise, chitin-treated and untreated water bath samples (W vs. CW) were statistically comparable on all day intervals ($p > 0.05$), with BI values increased from -4.99 to -3.22 and -4.72 to -2.89 respectively. In contrast, CP exhibited greater browning (-3.71) than P (-4.84) ($p < 0.05$) on Day 0, whereas no significant differences ($p > 0.05$) were observed on later storage intervals (Days 4, 8 and 12) with BI values of -3.75 and -4.44 for CP and P respectively on Day 12.

Table 4.3: Browning index of control and treated samples (W, P, CW, CP) during 12 days of storage.

	Browning Index			
	Day 0	**Day 4**	**Day 8**	**Day 12**
Control	-4.65 ± 0.18^{aAB}	-3.56 ± 0.51^{bB}	-2.01 ± 0.06^{cC}	-3.07 ± 0.40^{bcAB}
W	-4.99 ± 0.36^{aA}	-4.91 ± 0.13^{aA}	-4.62 ± 0.29^{aA}	-3.22 ± 0.63^{bAB}
P	-4.84 ± 0.63^{aA}	-4.28 ± 0.40^{aAB}	-4.14 ± 0.54^{aAB}	-4.44 ± 0.28^{aA}
CW	-4.72 ± 0.11^{aAB}	-4.11 ± 0.36^{aAB}	-4.16 ± 0.32^{aAB}	-2.89 ± 0.17^{bB}
CP	-3.71 ± 0.05^{aB}	-3.82 ± 0.57^{aAB}	-3.35 ± 0.31^{aB}	-3.75 ± 0.62^{aAB}

Values are expressed as mean ± standard deviation (n=3). Means with different lowercase superscripts indicate significant differences between days (within row) at $p < 0.05$. Means with different uppercase superscripts indicate significant differences between treatments (within column) at $p < 0.05$.

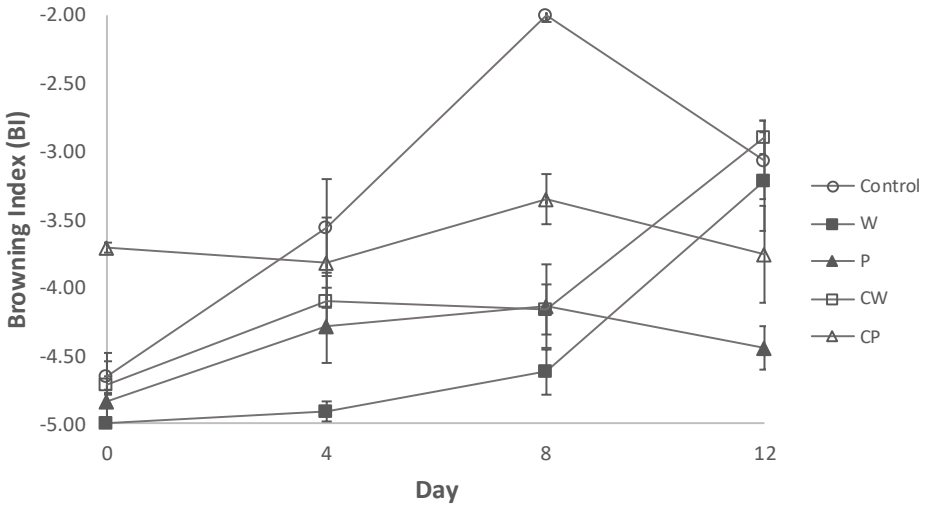

Figure 4.4: Changes in browning index of control and treated samples (W, P, CW, CP) during 12 days storage.

As shown in **Figure 4.5**, the Control sample showed the highest turbidity on Day 12, followed by CW and W. Both P and CP were noticeably clearer, displaying only a slight pale yellowish appearance.

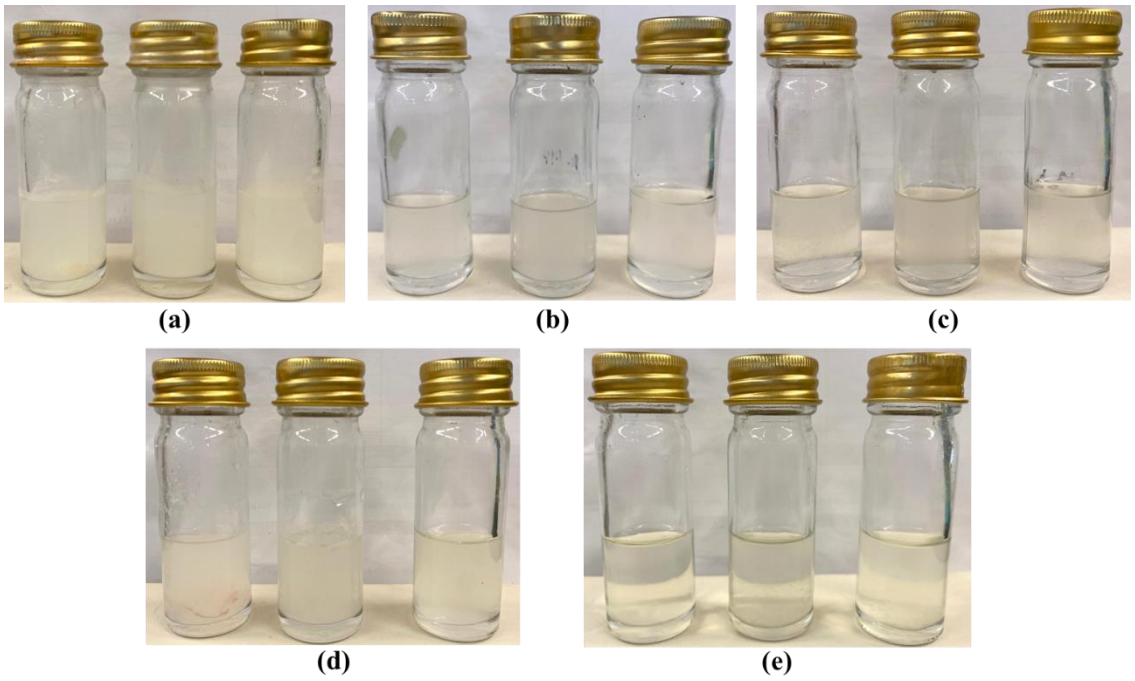

Figure 4.5: Visual appearance of MCW under different treatments on Day 12, shown in triplicates (n=3). (a) Control; (b) W; (c) P; (d) CW; (e) CP.

4.5 Relationship between TPC and Browning Index

Relationship between TPC and browning index for W, P, CW and CP during 12 days of storage is shown in **Figure 4.6**. For W, the browning index increased significantly from Day 0 to Day 12 ($p < 0.05$) with TPC maintained throughout the storage. For P, browning remained stable throughout 12 days of storage, with no significance difference in TPC between Day 0 and 12 ($p > 0.05$). For CW, browning increased significantly from Day 8 to Day 12, which corresponds to the increase of TPC from Day 8 to Day 12 ($p < 0.05$). For CP, browning remained consistent throughout storage period ($p > 0.05$) with TPC decreased from Day 0 to Day 8, followed by an increase by Day 12 ($p < 0.05$).

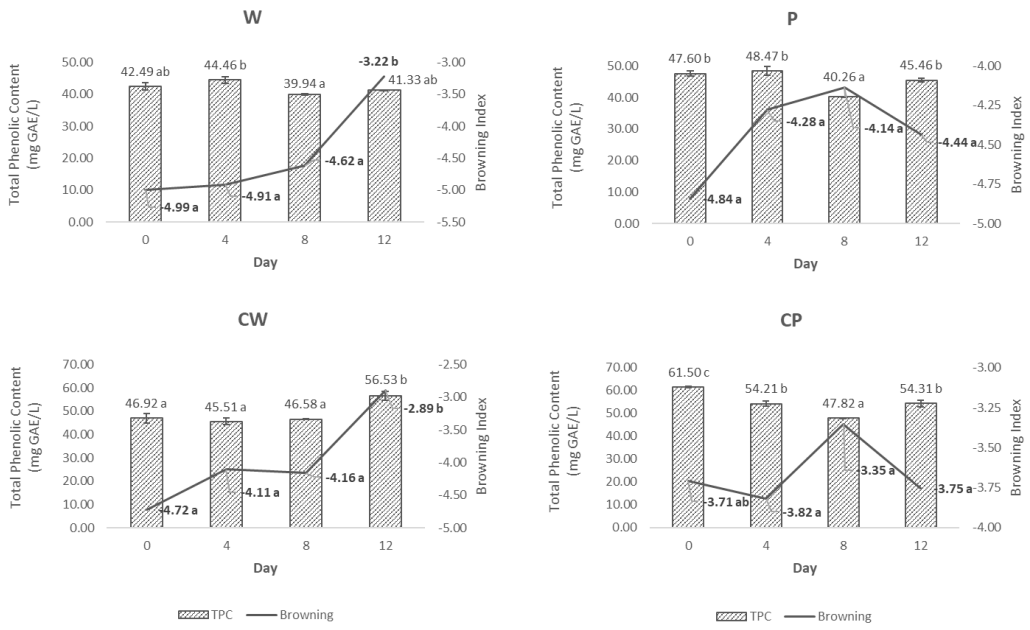

Figure 4.6: Relationship between TPC and browning index for W, P, CW, CP during 12 days storage. Means with different lowercase superscripts indicate significant differences between days at $p < 0.05$.

4.6 Determination of Total Plate Count

Figure 4.7 shows the spread plate results for total plate count on Day 12. No visible colonies were observed in W, P and CP. In contrast, CW plates showed confluent growth in two replicates, resulting in too numerous to count (TNTC).

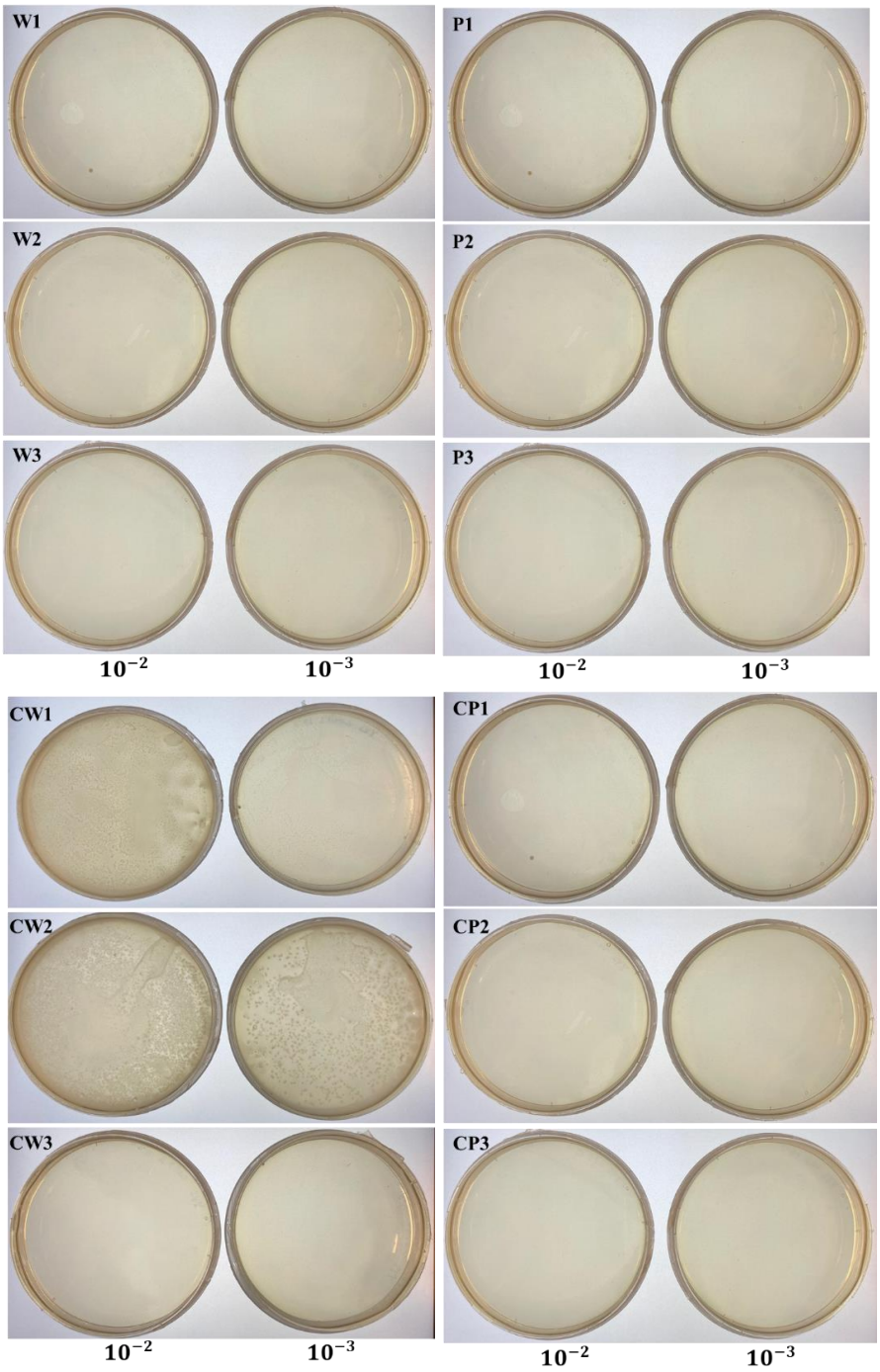

Figure 4.7: Spread plate observations of W, P, CW and CP on Day 12 after incubation at 37˚C for 24 hours, presented in triplicates (n=3).

CHAPTER 5

DISCUSSION

5.1 Polyphenol Oxidase and Peroxidase Inactivation Mechanisms

As shown in **Tables 4.1** and **4.2**, residual PPO activity was still detected, whereas POD activity was not detected in the control or in any of the treatments. According to Korbel, Brat and Servent (2013), a higher water activity (Aw) favours PPO thermal stability, whereas POD is more thermally stable at low Aw. Detudom, et al. (2023) considered, coconut water has high Aw as it contains up to 96% of water. The effect of Aw on the thermal stability of PPO and POD differ may be due to their different inactivation mechanisms. PPO is mainly inactivated by heat through alterations in its secondary structure, where a decrease in α-helix and β-turn content is accompanied by increases in β-sheet and random coil content, disruption of three-dimensional structure, and aggregation. In contrast, POD inactivation is typically associated with the dissociation of the prosthetic group from the holoenzyme and structural alterations in the apoenzyme. Generally, PPO is regarded as being more heat-stable than POD in fruit. In contrast POD is more thermostable than PPO in vegetables (Korbel, et al., 2014; Murtaza, et al., 2018).

5.2 Polyphenol Oxidase Activity

Residual PPO activity, ranging from 40 to 154.67 U/mL was detected in all treatments at Day 0, immediately after the treatments and throughout the storage

period, except for P and CP on Days 4, 8 and 12, where no PPO activity was detected **(Table 4.1)**. According to Mukhtar, et al. (2022), PPO activity was not detected in sugarcane juice after thermal pasteurization at 95℃ for 30 s. Campos, et al. (1996) also reported an inactivation temperature of 90℃ sufficiently inactivated PPO in coconut water within 550 s. Nevertheless, PPO still remained detected even though a longer heating duration was employed in this experiment. According to Kanjanapongkul and Baibua (2021), coconut water comprised two PPO isoenzymes, heat-sensitive and heat-stable group. When heat was applied, the heat-sensitive group denatured quickly, while the heat-stable group decreased at a comparatively slower rate. The lowest PPO activity was reached within 3 min at 90℃, with no further reduction during the subsequent 15 min of heating, indicating that the remaining PPO in coconut water was heat-resistant. Therefore, the residual PPO detected in W, P, CW and CP is suggested to be heat-stable.

According to Tan, et al. (2014), sucrose, a non-reducing sugar significantly increases in MCW as the coconut ages. In young coconut water, the main sugars are fructose, glucose and sucrose. As the coconut matures, sucrose account for around 90% of the total sugars, replacing much of the reducing sugars, fructose and glucose. Moreover, according to a study done by Liu, et al. (2021), sucrose, which is a disaccharide can act as a mild protective agent for PPO under certain conditions. For instance, high concentration of sucrose may promote thermostability of PPO, thus making it more resistant to heat-induces inactivation. This explains that there was still residual PPO activity detected in all treatments, with different extent.

Additionally, most studies have focused on the effect of heat treatment on enzyme activity in young coconut water, rather than in MCW. Since MCW undergoes substantial compositional changes during maturation, including increased mineral and protein contents, its chemical environment may differ significantly from that of young coconut water. These variations can influence enzyme stability as Tan, et al. (2014) also reported that the inactivation mechanisms of PPO and POD depend on sugar and salt contents.

5.2.1 Effect of Heat Treatment and Storage on PPO Activity

The control exhibited the highest PPO activity as it did not undergo any heat treatment (**Table 4.1**). Both W and P showed a significant reduction ($p < 0.05$), with P achieving more effective inactivation (**Table 4.1**), possibly due to the higher temperature reached within the same treatment time. Pressure cooking has been employed recently as an alternative to shorten cooking time and to preserve nutrient in food. According to Rocca-Poliméni, Flick and Vasseur (2011), pressure cooker works by keeping food and water inside a vessel and heat it. Steam that is built up inside the vessel increases internal pressure and therefore raises the boiling point of coconut water. Consequently, MCW (P) was being processed at a higher temperature compared to conventional water bath heat treatment (WB). Moreover, efficient heat transfer can be achieved in a pressure cooker through conduction (from the hotplate or heating element to food), convection (movement of steam and liquid) and condensation (steam condensing on cooler surfaces) (Rocca-Poliméni, Flick and Vasseur, 2011).

Enzymes are generally vulnerable to heat as they are proteins with three-dimensional structure and specific active sites. These structures and active sites can be altered when high heat is introduced, resulting in loss of catalytic function (Wang, et al., 2024; Schokker and Boekel, 1997; Tsukidate, et al., 2023). As higher temperature is achieved in pressure cooking, the extent of enzyme inactivation in P would therefore be more pronounced. A study carried out by Murtaza, et al. (2018) stated that heat treatment could increase the natural tendency of PPO to aggregate by encouraging interactions that lead to small units clumping into bigger particles, which may block active sites and decrease activity. Heat can also cause dissociation, which breaks big aggregates into smaller pieces and may increase activity by momentarily exposing additional catalytic sites. This effect only occurs under high temperature, short time treatment, whereas the long heating duration (20 min) used in the experiment likely prevented it from happening. Moreover, PPO binds with its substrate through various interactions, including hydrogen bonds, hydrophobic interactions and π-bond interactions. Under high heat, hydrophobic and π-bond interactions are disrupted, thereby weakening the binding ability of PPO (Qu, et al., 2025).

As shown in **Table 4.1**, PPO activity decreased from Day 0 to Day 12 in control, W, P, CW and CP. This finding was supported by Ranveer, Pawar and Sakhale (2010) as PPO in raisins showed gradual reduction upon storage at room temperature. As PPO requires oxygen to support its activity, it may gradually lose activity during

storage under appropriate condition in the absence of oxygen. The increase in PPO activity in the control from Day 4 to Day 8 may be due to the presence of microorganisms, which could have altered the chemical environment and enzyme activity in the sample (**Table 4.1**).

5.3 Effect of Heat Treatments on POD Activity

There was no POD activity observed in any of the treatments, including the control on Day 0. This result contrasts with previous studies, where POD activity was generally detected. This discrepancy may be due to the fact that young coconut water is commonly assessed for enzymatic activities, where its composition is different from that of mature coconut water (Tan, et al., 2014). Also, coconut with different variety used across studies may also account for the variations.

Kanjanapongkul and Baibua (2021) reported, a complete loss of POD activity in young coconut water heated at 90°C for three minutes. In addition, Campos, et al. (1996) found that POD in young coconut water was completely inactivated within 310 s at 90°C. POD was found to be more heat-senstitive than PPO as it was inactivated at shorter holding times. Moreover, enzyme activity decreases as the coconut matures. Therefore, POD may either be absent or present in very small amounts in MCW, making it undetectable (Garcia, et al., 2007).

5.4 Total Phenolic Content

5.4.1 Effect of Heat Treatments and Storage on TPC

According to Mahayothee, et al. (2015), coconut at 225 days after pollination showed a TPC of around 5.80 to 6.68 mg GAE/ 100 mL, which corresponds to our findings, where the TPC of the control on Day 0 was 53.50 ± 0.82 mg GAE/ L. When compared to control, both heat-treated MCW (W and P) and chitin with water bath-treated MCW (CW) show a reduction in TPC. The reduction was significant in W and CW but insignificant in P, and there is no significant difference between the two thermal treated treatments (W and P) (**Table 4.2**). Catechin, epicatechin and salicylic acid constitute the majority of the overall phenolic content of coconut water (Mahayothee, et al., 2016; Halim, et al., 2023; Syahputri, Santoso and Supriyanto, 2021). Upon heat treatment, phenolic content decreases mainly due to several reactions occurred. For instance, degradation, oxidation or polymerization (Maghsoudlou, Asghari Ghajari and Tavasoli, 2019). Catechin is heat-sensitive and it is prone to oxidation or degradation during thermal treatment, specifically 90°C for 100 seconds and above as suggested by Tan, et al. (2014) may lead to formation of browning compound. This causes a reduction in catechin content (Magangana, et al., 2022; Tan, et al., 2014). Epicatechin behaves similarly as catechin, where a reduction was observed in raw pomegranate peel after blanching at 80°C for 3 min (Magangana, et al., 2022). CP exhibited a significantly higher TPC than the control after treatment (**Table 4.2**) which may be attributed to the unclear interaction between chitin and MCW as well as the reactions induced by the prolonged shaking process (4 h).

According to Kumar, et al. (2023), the TPC in pomelo juice and kinnow juice did not change significantly after heat treatment at various temperatures, for instance, 70℃, 80℃, 90℃ and 100℃. However, the duration of the treatment time had a greater effect. In pomelo juice, the change in TPC was minimal during the first 30 min of treatment, but it decreased drastically after 45 min. Similarly, kinnow juice exhibited a comparable pattern, where TPC decreased significantly only after 15 min of treatment. Shourove, et al. (2002) also stated that changes in TPC in star fuit juice depend on the treatment conditions and duration. For instance, TPC increased when the juice was treated at 70℃ for 10 min, but decreased when subjected to heat treatment at 90℃ for 40 min.

W and P showed a stable trend in TPC while CW experienced an increase from Day 0 to Day 12 and CP experienced a reduction in TPC during storage (**Table 4.2**). The increase in TPC in CW may be due to the presence of bacteria in the sample, as the water bath treatment may not have been sufficient to eliminate the higher microbial load introduced during addition of chitin compared to pressure-cook treatment. According to Zhao, et al. (2021), microorganism-induced fermentation increased TPC as new compounds were synthesized. This is also attributed to the hydrolytic reaction caused by microbial activity (Hur, et al., 2014). This is likely to occur as the samples were tightly sealed with parafilm and were maintained in an anaerobic environment. TPC declined in CP is corroborated with Goh, et al. (2012), which showed a progressive decline in TPC in pomegranate juice that had been thermally

and UV-treated after two weeks of storage. Numerous studies have demonstrated that heat treatment can either increase or decrease TPC, depending on the sample type and treatment parameters (Shourove, et al., 2020).

5.4.2 Effect of Chitin Treatment on TPC

According to Cosme and Vilela (2021), chitin possesses a dual role where unstable phenolic compound like catechins can be reduced, while in some cases, major phenolic loss is not observed during the clarification process of green tea using chitosan. Chitin and its deacetylated form, chitosan reacts similarly but differ in solubility extent (Cosme and Vilela, 2021). According to Spagna, et al. (1996), the removal of phenolic compound was less effective with chitin compared to chitosan. This may be due to the low solubility of chitin in common organic and dilute aqueous solvents, therefore compromising its ability to remove phenolic compounds as seen in **Table 4.2** (Zargar, Asghari and Dashti, 2014) where both chitin-treated samples (CW and CP) exhibited a higher TPC than untreated samples. In addition, phenol absorption by chitin is significantly influenced by the initial pH of the adsorption medium. For instance, protonation of chitin's functional groups produces positive charges that electrostatically attract negatively charged phenolate ions where adsorption is most significant at low pH values (pH 1). The surface of chitin becomes increasingly negatively charged when pH rises, which decreases attraction and therefore, adsorption efficiency decreases (Dursun and Kalayci, 2005). For instance, the pH of mature coconut ranges from 5.58 to 5.84, which is

considered less acidic and closer to neutral (Tan, et al., 2014; Mahayothee, et al., 2016).

Moreover, the Folin-Ciocalteu assay does not specifically quantify phenolic compounds; instead, non-phenolic reducing compound that may be formed during heat treatment may cause an inflation in Folin reading and leads to an overestimation of phenolic compounds (Pérez, Dominguez-López and Lamuela-Raventós, 2023).

5.5 Relationship between PPO activity, TPC and Browning

As shown in **Table 4.1**, control exhibited the highest PPO activity in all time interval, this is correlated with the increase of browning (**Table 4.3**). Control did not undergo any treatment. Thus, microorganisms, enzymes and naturally present phenolic compounds still remained in it. PPO is regarded as the main enzyme causing enzymatic browning in fruits, vegetables as well as coconut water (Qu, et al., 2025). According to Concellón, Añón and Chaves (2004), PPO oxidizes phenolic compounds to form brown-coloured quinones, resulting in the browning phenomenon. Quinone can then bind covalently to PPO and therefore reduce its activity (Vámos-Vigyázó and Haard, 1981). This may explain why PPO activity decreased in the control, W and CW but browning continued to increase during storage. Vámos-Vigyázó and Haard (1981) also stated that PPO activity in apples and strawberries decreased during frozen and cold storage. For P and CP, browning did not increase during storage (**Table 4.3**), which can be due to the more effective

inactivation of PPO activity achieved by the pressure-cook heat treatment. The absence of PPO activity in P and CP on Days 4, 8 and 12 further supports the observation that no browning developed during storage (**Table 4.1**).

Based on **Figure 4.6**, both W and CW show a significant increase in browning from Day 8 to Day 12. During this period, TPC remained relatively stable in W while an increase was observed in CW. Increased TPC in CW may be due to unequal buildup of particular phenolic compounds over time as suggested by Wang, et al. (2025). On the other hand, browning increased may also be due to the availability of PPO where phenolic compounds (substrate) were oxidized in the presence of residual oxygen in the sample to produce brown-coloured quinones (Concellón, Añón and Chaves, 2004). Consequently, the phenolic compounds were reduced, and browning was observed. P and CP exhibited insignificant browning as well as stable trend of TPC over 12 days storage. It can be confirmed that browning developed during storage was due to an enzymatic reaction. PPO activity has been reduced to undetectable levels for P and CP on Day 4, 8 and 12, which means that enzymatic browning could not take place even when the substrate is present. CP exhibited a significantly higher browning index than P on Day 0, possibly due to the incorporation of oxygen during the shaking process, which facilitates oxygen transfer (Grassie, 2025). This, in turn, may have led to the oxidation of TPC, resulting in the formation of brown-coloured quinones. According to Garcia, et al. (2007), certain phenolic compounds may function as synergists or inhibitors of PPO but do not contribute to enzymatic browning.

5.6 Total Plate Count

Based on **Figure 4.7**, W, P and CP have no colonies observed in agar plates on Day 12 while there was confluent growth of colonies observed in CW. This observation demonstrates that both water bath and pressure-cooking treatment for 20 min were successful in eliminating bacteria in MCW. However, CW exhibited confluent growth of colonies, which may be due to contaminants present in chitin, that may be introduced unintentionally into MCW samples. Additionally, this may be linked to the lower efficiency of the water bath treatment when a higher bacterial load is present, possibly due to contamination that occurred during the addition of chitin. No colonies were detected in CP, in which the MCW underwent pressure-cook heat treatment.

According to Tongdonyod, et al. (2023), the maximum acceptable numbers for total plate count in ready-to-drink coconut water beverage is 1×10^4 CFU/mL. Based on this standard, W, P and CP met the requirements for safe consumption. A study carried out by Gunathunga, Abeywickrema and Navaratne (2018) stated that heat treatment of 85℃ for 60s was sufficient to preserve the microbial quality of coconut water that stored at 4±2℃ for up to 2 weeks. On the other hand, Tongdonyod, et al. (2023) also reported that less than 1 log CFU/mL was observed in coconut water treated in 85℃ water bath for 10 minutes over 35 days of storage.

5.7 Feasibility of Chitin Application in MCW Processing

According to **Table 4.3**, P and CP did not experience significant browning throughout 12 days storage with P having lower initial browning index while W and CW experienced browning from Day 8 to Day 12 with no significant difference among them. With these observations, it can be said that chitin treatment did not significantly mitigate the browning issues encountered in coconut water. Instead, chitosan, the deacetylated form of chitin can serve as an alternative for removing phenolic compounds in coconut water as it has been documented to be more effective than chitin in its removal. Also, chitin is primarily obtained from crustacean shells, which may present allergenic risk for individuals with seafood sensitivities. As a result, only fungal-derived chitin or chitosan is permitted to be applied in food industry (Cosme and Vilela, 2022).

5.8 Limitations and Future Recommendations Study

Chitin application in coconut water requires further investigation as there are limited studies on its use for treating coconut water, fruit juices or beverages to remove phenolic compounds. Its effectiveness is highly dependent on the chemical environments of the medium, for instance pH, temperature and initial phenolic concentration can influence its performance (Dursun and Kalayci, 2005). While chitin has been effectively applied in wastewater treatment to remove phenolic compounds, it is not widely used in the food industry, mainly due to its poor solubility in aqueous solutions compared to its deacetylated form, chitosan and the lack of documentation regarding its compatibility with different beverages.

Chitosan is more commonly applied in beverages for purposes such as clarification and stabilization, owing to its better solubility. However, future studies should focus on chitosan for removing phenolic compounds in coconut water. Despite its improved solubility, separating chitosan from the adsorption medium may present challenges, as it can dissolve into the solvent. Therefore, further research is required to confirm its practical applicability (Cosme and Vilela, 2021; Spagna, et al., 1996). Moreover, most existing studies have primarily focused on young coconut water rather than mature coconut water, where the composition changes notably during maturation. For instance, differences can be observed in total soluble solids, titratable acidity, sugar content, minerals content and TPC (Tan, et al., 2014). These compositional variations may lead to different outcomes as the chemical environment significantly influences chitin performance and there is also limited information on how mature coconut water interacts with these compounds.

In addition, thermal treatment may induce undesirable changes in MCW, including alterations in nutritional value and discolouration caused by the Maillard reaction, a common process in which reducing sugars and amino acids present in coconut water interact. To address this limitation, non-thermal treatments have emerged as alternative approaches. For instance, the ultrafiltration technique has been shown to preserve most of the chemical properties of coconut water while reducing PPO and POD activity and improving clarity. However, TPC and antioxidant properties are not well retained, indicating the need for further investigation (Syahputri, Santoso and Supriyanto, 2021). On the other hand, combined vacuum-microwave

treatment has also been recognized as promising alternative, as it preserves the sensory properties, shelf-life stability and physicochemical characteristics of coconut water at high quality, making it preferable to the fresh sample (Juli, et al., 2023).

Lastly, sensory evaluation can be included to assess the feasibility of the treatments mentioned above. This would help confirm whether mature coconut water remains palatable for consumption after undergoing heat treatment and chitin application.

CHAPTER 6

CONCLUSIONS

In conclusion, the objectives of this study were successfully achieved. Pressure-cook treatment was found to be more effective in inactivating enzymatic activity, particularly polyphenol oxidase (PPO), as the higher temperature and slightly elevated pressure in the pressure cooker enhanced enzyme deactivation. Peroxidase (POD) was not detected in any of the samples, and the absence of POD in the control contrasts with findings from most other studies. This discrepancy may be attributed to differences in coconut cultivars, as mature coconuts generally exhibit lower enzymatic activity than young coconuts. Therefore, POD may not be present in MCW. Regarding total phenolic content (TPC), W, P and CW showed a reduction, whereas CP exhibited an increase on Day 0. TPC reduction may be due to the degradation of heat-sensitive phenolic compounds naturally present in coconut water, particularly catechin and epicatechin. During storage, W and P maintained relatively stable TPC levels, while CW increased and CP decreased. The increase in CW may be attributed to contamination, which could have led to the synthesis of new compounds induced by microbial fermentation. Furthermore, TPC in chitin-treated samples (CW and CP) was higher than in untreated samples (W and P), likely due to the influence of the pH of the medium and the solubility of chitin. The control exhibited the highest PPO activity, which corresponded to its increased browning during storage, as it was not subjected to any treatment. In contrast, P and CP showed no significant browning during storage due to more effective PPO

inactivation, with activity reduced to undetectable levels on Days 4, 8 and 12. This was further supported by the constant TPC values observed throughout storage. Meanwhile, browning increased in W and CW during storage, likely due to residual PPO activity and the availability of phenolic compounds serving as substrates. Overall, the findings suggest that enzymatic activity is the main factor driving browning in MCW during storage. However, as P and CP showed no significant difference in browning index on Day 12 and W and CW displayed similar behaviour, it is suggested that chitin may not be fully compatible with coconut water in functioning as a phenolic compound removal agent. For microbial quality testing, three out of the four treatments (W, P and CP) showed no detectable colonies on PCA agar on Day 12, whereas confluent growth was observed in CW. This may be due to unintentional contamination introduced through chitin and the lower efficiency of the water bath treatment.

This study faced several limitations, including the limited research on chitin application in coconut water, the poorly characterized composition of MCW and potential quality deterioration from thermal treatment. Future work should consider using chitosan, exploring non-thermal preservation methods and conducting sensory evaluations to assess consumer acceptance.

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APPENDICES

APPENDIX A

Raw data of the colour parameters, *L, *a and *b values

Table A.1: Lightness (*L) changes over 12 days storage.

	*L			
	Day 0	**Day 4**	**Day 8**	**Day 12**
Control	46.44 ± 0.14	45.90 ± 0.37	45.35 ± 0.33	47.51 ± 0.05
WB	46.07 ± 0.28	47.03 ± 0.22	47.11 ± 0.07	48.59 ± 0.08
P	46.54 ± 0.15	46.81 ± 0.01	47.34 ± 0.43	49.02 ± 0.08
CW	46.18 ± 0.21	47.03 ± 0.45	46.99 ± 0.38	47.73 ± 0.13
CP	45.43 ± 0.08	46.71 ± 0.32	46.25 ± 0.04	47.47 ± 0.32

Table A.2: Redness (*a) changes over 12 days storage.

	*a			
	Day 0	**Day 4**	**Day 8**	**Day 12**
Control	0.54 ± 0.01	0.51 ± 0.05	0.45 ± 0.05	0.43 ± 0.03
WB	0.58 ± 0.03	0.59 ± 0.00	0.50 ± 0.05	0.50 ± 0.01
P	0.54 ± 0.05	0.51 ± 0.07	0.50 ± 0.01	0.45 ± 0.05
CW	0.55 ± 0.02	0.51 ± 0.03	0.45 ± 0.05	0.45 ± 0.06
CP	0.53 ± 0.02	0.51 ± 0.01	0.45 ± 0.04	0.45 ± 0.05

Table A.3: Yellowness (*b) changes over 12 days storage.

	*b			
	Day 0	**Day 4**	**Day 8**	**Day 12**
Control	-2.69 ± 0.08	-2.09 ± 0.25	-1.29 ± 0.07	-1.84 ± 0.21

WB	-2.88 ± 0.18	-2.90 ± 0.06	-2.68 ± 0.14	-2.02 ± 0.33
P	-2.77 ± 0.32	-2.47 ± 0.21	-2.43 ± 0.29	-2.62 ± 0.14
CW	-2.72 ± 0.06	-2.44 ± 0.17	-2.40 ± 0.17	-1.79 ± 0.05
CP	-1.98 ± 0.33	-2.26 ± 0.28	-1.98 ± 0.17	-2.23 ± 0.32

APPENDIX B

Two-way ANOVA and Tukey's test of PPO activity, TPC and browning index across storage days

Table B.1: Two-way ANOVA of PPO activtiy.

Tests of Between-Subjects Effects

Dependent Variable: PPO

Heat trt	Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Control	Corrected Model	113219.556[a]	3	37739.852	136.081	<.001
	Intercept	960024.242	1	960024.242	3461.626	<.001
	Storage_time	113219.556	3	37739.852	136.081	<.001
	Error	1386.667	5	277.333		
	Total	1038768.000	9			
	Corrected Total	114606.222	8			
W	Corrected Model	21936.889[b]	3	7312.296	22.587	.002
	Intercept	51968.242	1	51968.242	160.528	<.001
	Storage_time	21936.889	3	7312.296	22.587	.002
	Error	1618.667	5	323.733		
	Total	89776.000	9			
	Corrected Total	23555.556	8			
P	Corrected Model	5504.727[c]	3	1834.909	178.394	<.001
	Intercept	2242.667	1	2242.667	218.037	<.001
	Storage_time	5504.727	3	1834.909	178.394	<.001
	Error	72.000	7	10.286		
	Total	6800.000	11			
	Corrected Total	5576.727	10			
CW	Corrected Model	9880.000[d]	3	3293.333	68.611	<.001
	Intercept	49928.000	1	49928.000	1040.167	<.001
	Storage_time	9880.000	3	3293.333	68.611	<.001
	Error	192.000	4	48.000		
	Total	60000.000	8			
	Corrected Total	10072.000	7			
CP	Corrected Model	2560.000[e]	3	853.333	160.000	<.001
	Intercept	960.000	1	960.000	180.000	<.001
	Storage_time	2560.000	3	853.333	160.000	<.001
	Error	32.000	6	5.333		
	Total	3232.000	10			
	Corrected Total	2592.000	9			

a. R Squared = .988 (Adjusted R Squared = .981)

b. R Squared = .931 (Adjusted R Squared = .890)

c. R Squared = .987 (Adjusted R Squared = .982)

d. R Squared = .981 (Adjusted R Squared = .967)

e. R Squared = .988 (Adjusted R Squared = .981)

Table B.2: Tukey's test of PPO activity.

Multiple Comparisons

Dependent Variable: PPO

Heat trt		(I) Storage	(J) Storage	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Control	Tukey HSD	day 0	day 4	268.0000*	16.65333	<.001	206.5507	329.4493
			day 8	196.0000*	16.65333	<.001	134.5507	257.4493
			day 12	289.3333*	15.20234	<.001	233.2380	345.4286
		day 4	day 0	-268.0000*	16.65333	<.001	-329.4493	-206.5507
			day 8	-72.0000*	16.65333	.027	-133.4493	-10.5507
			day 12	21.3333	15.20234	.548	-34.7620	77.4286
		day 8	day 0	-196.0000*	16.65333	<.001	-257.4493	-134.5507
			day 4	72.0000*	16.65333	.027	10.5507	133.4493
			day 12	93.3333*	15.20234	.006	37.2380	149.4286
		day 12	day 0	-289.3333*	15.20234	<.001	-345.4286	-233.2380
			day 4	-21.3333	15.20234	.548	-77.4286	34.7620
			day 8	-93.3333*	15.20234	.006	-149.4286	-37.2380
W	Tukey HSD	day 0	day 4	114.6667*	16.42491	.004	54.0602	175.2732
			day 8	104.6667*	16.42491	.005	44.0602	165.2732
			day 12	90.6667*	16.42491	.010	30.0602	151.2732
		day 4	day 0	-114.6667*	16.42491	.004	-175.2732	-54.0602
			day 8	-10.0000	17.99259	.941	-76.3911	56.3911
			day 12	-24.0000	17.99259	.583	-90.3911	42.3911
		day 8	day 0	-104.6667*	16.42491	.005	-165.2732	-44.0602
			day 4	10.0000	17.99259	.941	-56.3911	76.3911
			day 12	-14.0000	17.99259	.861	-80.3911	52.3911
		day 12	day 0	-90.6667*	16.42491	.010	-151.2732	-30.0602
			day 4	24.0000	17.99259	.583	-42.3911	90.3911
			day 8	14.0000	17.99259	.861	-52.3911	80.3911
P	Tukey HSD	day 0	day 4	58.0000*	2.92770	<.001	48.3088	67.6912
			day 8	58.0000*	2.92770	<.001	48.3088	67.6912
			day 12	58.0000*	2.92770	<.001	48.3088	67.6912
		day 4	day 0	-58.0000*	2.92770	<.001	-67.6912	-48.3088
			day 8	.0000	2.61861	1.000	-8.6680	8.6680
			day 12	.0000	2.61861	1.000	-8.6680	8.6680
		day 8	day 0	-58.0000*	2.92770	<.001	-67.6912	-48.3088
			day 4	.0000	2.61861	1.000	-8.6680	8.6680
			day 12	.0000	2.61861	1.000	-8.6680	8.6680
		day 12	day 0	-58.0000*	2.92770	<.001	-67.6912	-48.3088
			day 4	.0000	2.61861	1.000	-8.6680	8.6680
			day 8	.0000	2.61861	1.000	-8.6680	8.6680
CW	Tukey HSD	day 0	day 4	32.0000*	6.92820	.033	3.7963	60.2037
			day 8	76.0000*	6.92820	.001	47.7963	104.2037
			day 12	88.0000*	6.92820	<.001	59.7963	116.2037
		day 4	day 0	-32.0000*	6.92820	.033	-60.2037	-3.7963
			day 8	44.0000*	6.92820	.011	15.7963	72.2037
			day 12	56.0000*	6.92820	.004	27.7963	84.2037
		day 8	day 0	-76.0000*	6.92820	.001	-104.2037	-47.7963
			day 4	-44.0000*	6.92820	.011	-72.2037	-15.7963
			day 12	12.0000	6.92820	.415	-16.2037	40.2037
		day 12	day 0	-88.0000*	6.92820	<.001	-116.2037	-59.7963
			day 4	-56.0000*	6.92820	.004	-84.2037	-27.7963
			day 8	-12.0000	6.92820	.415	-40.2037	16.2037
CP	Tukey HSD	day 0	day 4	40.0000*	2.10819	<.001	32.7021	47.2979
			day 8	40.0000*	2.10819	<.001	32.7021	47.2979
			day 12	40.0000*	2.30940	<.001	32.0055	47.9945
		day 4	day 0	-40.0000*	2.10819	<.001	-47.2979	-32.7021
			day 8	.0000	1.88562	1.000	-6.5275	6.5275
			day 12	.0000	2.10819	1.000	-7.2979	7.2979
		day 8	day 0	-40.0000*	2.10819	<.001	-47.2979	-32.7021
			day 4	.0000	1.88562	1.000	-6.5275	6.5275
			day 12	.0000	2.10819	1.000	-7.2979	7.2979
		day 12	day 0	-40.0000*	2.30940	<.001	-47.9945	-32.0055
			day 4	.0000	2.10819	1.000	-7.2979	7.2979
			day 8	.0000	2.10819	1.000	-7.2979	7.2979

Based on observed means.
The error term is Mean Square(Error) = 5.333.

*. The mean difference is significant at the 0.05 level.

Table B.3: Two-way ANOVA of TPC results.

Tests of Between-Subjects Effects

Dependent Variable: TPC

Heat trt	Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Control	Corrected Model	565.203[a]	3	188.401	41.086	<.001
	Intercept	46979.872	1	46979.872	10245.362	<.001
	Storage_time	565.203	3	188.401	41.086	<.001
	Error	32.098	7	4.585		
	Total	50767.500	11			
	Corrected Total	597.301	10			
W	Corrected Model	28.009[b]	3	9.336	5.557	.036
	Intercept	16979.656	1	16979.656	10106.353	<.001
	Storage_time	28.009	3	9.336	5.557	.036
	Error	10.081	6	1.680		
	Total	17866.583	10			
	Corrected Total	38.090	9			
P	Corrected Model	117.083[c]	3	39.028	17.381	.001
	Intercept	22030.807	1	22030.807	9811.344	<.001
	Storage_time	117.083	3	39.028	17.381	.001
	Error	15.718	7	2.245		
	Total	22656.984	11			
	Corrected Total	132.801	10			
CW	Corrected Model	170.482[d]	3	56.827	8.001	.016
	Intercept	22939.267	1	22939.267	3229.661	<.001
	Storage_time	170.482	3	56.827	8.001	.016
	Error	42.616	6	7.103		
	Total	23588.872	10			
	Corrected Total	213.098	9			
CP	Corrected Model	233.244[e]	3	77.748	27.331	<.001
	Intercept	31633.079	1	31633.079	11120.022	<.001
	Storage_time	233.244	3	77.748	27.331	<.001
	Error	19.913	7	2.845		
	Total	33602.117	11			
	Corrected Total	253.156	10			

a. R Squared = .946 (Adjusted R Squared = .923)

b. R Squared = .735 (Adjusted R Squared = .603)

c. R Squared = .882 (Adjusted R Squared = .831)

d. R Squared = .800 (Adjusted R Squared = .700)

e. R Squared = .921 (Adjusted R Squared = .888)

Table B.4: Tukey's test of TPC results.

Multiple Comparisons

Dependent Variable: TPC

Heat trt		(I) Storage	(J) Storage	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Control	Tukey HSD	day 0	day 4	-17.98517*	1.954797	<.001	-24.45586	-11.51447
			day 8	-20.40117*	1.954797	<.001	-26.87186	-13.93047
			day 12	-13.06117*	1.954797	.001	-19.53186	-6.59047
		day 4	day 0	17.98517*	1.954797	<.001	11.51447	24.45586
			day 8	-2.41600	1.748423	.547	-8.20356	3.37156
			day 12	4.92400	1.748423	.095	-.86356	10.71156
		day 8	day 0	20.40117*	1.954797	<.001	13.93047	26.87186
			day 4	2.41600	1.748423	.547	-3.37156	8.20356
			day 12	7.34000*	1.748423	.017	1.55244	13.12756
		day 12	day 0	13.06117*	1.954797	.001	6.59047	19.53186
			day 4	-4.92400	1.748423	.095	-10.71156	.86356
			day 8	-7.34000*	1.748423	.017	-13.12756	-1.55244
W	Tukey HSD	day 0	day 4	-1.96883	1.183250	.415	-6.06490	2.12724
			day 8	2.55200	1.296186	.295	-1.93502	7.03902
			day 12	1.16650	1.183250	.763	-2.92957	5.26257
		day 4	day 0	1.96883	1.183250	.415	-2.12724	6.06490
			day 8	4.52083*	1.183250	.033	.42476	8.61690
			day 12	3.13533	1.058331	.090	-.52830	6.79897
		day 8	day 0	-2.55200	1.296186	.295	-7.03902	1.93502
			day 4	-4.52083*	1.183250	.033	-8.61690	-.42476
			day 12	-1.38550	1.183250	.664	-5.48157	2.71057
		day 12	day 0	-1.16650	1.183250	.763	-5.26257	2.92957
			day 4	-3.13533	1.058331	.090	-6.79897	.52830
			day 8	1.38550	1.183250	.664	-2.71057	5.48157
P	Tukey HSD	day 0	day 4	-.87483	1.367919	.916	-5.40287	3.65320
			day 8	7.34017*	1.367919	.004	2.81213	11.86820
			day 12	2.13850	1.367919	.454	-2.38953	6.66653
		day 4	day 0	.87483	1.367919	.916	-3.65320	5.40287
			day 8	8.21500*	1.223504	.001	4.16500	12.26500
			day 12	3.01333	1.223504	.151	-1.03666	7.06333
		day 8	day 0	-7.34017*	1.367919	.004	-11.86820	-2.81213
			day 4	-8.21500*	1.223504	.001	-12.26500	-4.16500
			day 12	-5.20167*	1.223504	.015	-9.25166	-1.15167
		day 12	day 0	-2.13850	1.367919	.454	-6.66653	2.38953
			day 4	-3.01333	1.223504	.151	-7.06333	1.03666
			day 8	5.20167*	1.223504	.015	1.15167	9.25166
CW	Tukey HSD	day 0	day 4	1.40967	2.176034	.913	-6.12313	8.94247
			day 8	.34000	2.432880	.999	-8.08193	8.76193
			day 12	-9.61200*	2.432880	.029	-18.03393	-1.19007
		day 4	day 0	-1.40967	2.176034	.913	-8.94247	6.12313
			day 8	-1.06967	2.432880	.969	-9.49159	7.35226
			day 12	-11.02167*	2.432880	.016	-19.44359	-2.59974
		day 8	day 0	-.34000	2.432880	.999	-8.76193	8.08193
			day 4	1.06967	2.432880	.969	-7.35226	9.49159
			day 12	-9.95200*	2.665086	.037	-19.17776	-.72624
		day 12	day 0	9.61200*	2.432880	.029	1.19007	18.03393
			day 4	11.02167*	2.432880	.016	2.59974	19.44359
			day 8	9.95200*	2.665086	.037	.72624	19.17776
CP	Tukey HSD	day 0	day 4	7.29133*	1.377122	.005	2.73284	11.84983
			day 8	13.68367*	1.539669	<.001	8.58711	18.78022
			day 12	7.19433*	1.377122	.005	2.63584	11.75283
		day 4	day 0	-7.29133*	1.377122	.005	-11.84983	-2.73284
			day 8	6.39233*	1.539669	.017	1.29578	11.48889
			day 12	-.09700	1.377122	1.000	-4.65550	4.46150
		day 8	day 0	-13.68367*	1.539669	<.001	-18.78022	-8.58711
			day 4	-6.39233*	1.539669	.017	-11.48889	-1.29578
			day 12	-6.48933*	1.539669	.016	-11.58589	-1.39278
		day 12	day 0	-7.19433*	1.377122	.005	-11.75283	-2.63584
			day 4	.09700	1.377122	1.000	-4.46150	4.65550
			day 8	6.48933*	1.539669	.016	1.39278	11.58589

Based on observed means.
The error term is Mean Square(Error) = 1.782.

*. The mean difference is significant at the 0.05 level.

Table B.5: Two-way ANOVA of browning index results.

Tests of Between-Subjects Effects

Dependent Variable: Browning index

Heat trt	Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Control	Corrected Model	8.800[a]	3	2.933	30.117	.001
	Intercept	96.167	1	96.167	987.371	<.001
	Storage_time	8.800	3	2.933	30.117	.001
	Error	.487	5	.097		
	Total	117.475	9			
	Corrected Total	9.287	8			
W	Corrected Model	6.142[b]	3	2.047	13.025	.002
	Intercept	236.119	1	236.119	1502.191	<.001
	Storage_time	6.142	3	2.047	13.025	.002
	Error	1.257	8	.157		
	Total	243.519	12			
	Corrected Total	7.399	11			
P	Corrected Model	.815[c]	3	.272	1.127	.401
	Intercept	208.860	1	208.860	865.955	<.001
	Storage_time	.815	3	.272	1.127	.401
	Error	1.688	7	.241		
	Total	219.176	11			
	Corrected Total	2.504	10			
CW	Corrected Model	4.044[d]	3	1.348	17.926	.001
	Intercept	168.081	1	168.081	2235.190	<.001
	Storage_time	4.044	3	1.348	17.926	.001
	Error	.526	7	.075		
	Total	186.540	11			
	Corrected Total	4.570	10			
CP	Corrected Model	.386[e]	3	.129	.549	.665
	Intercept	142.951	1	142.951	610.109	<.001
	Storage_time	.386	3	.129	.549	.665
	Error	1.640	7	.234		
	Total	149.085	11			
	Corrected Total	2.026	10			

a. R Squared = .948 (Adjusted R Squared = .916)

b. R Squared = .830 (Adjusted R Squared = .766)

c. R Squared = .326 (Adjusted R Squared = .037)

d. R Squared = .885 (Adjusted R Squared = .835)

e. R Squared = .190 (Adjusted R Squared = -.157)

Table B.6: Tukey's test of browning index results.

Multiple Comparisons

Dependent Variable: Browning index

Heat trt		(I) Storage	(J) Storage	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Control	Tukey HSD	day 0	day 4	-1.0880*	.28489	.044	-2.1393	-.0368
			day 8	-2.6430*	.28489	<.001	-3.6943	-1.5918
			day 12	-1.5830*	.28489	.010	-2.6343	-.5318
		day 4	day 0	1.0880*	.28489	.044	.0368	2.1393
			day 8	-1.5550*	.31208	.015	-2.7066	-.4034
			day 12	-.4950	.31208	.461	-1.6466	.6566
		day 8	day 0	2.6430*	.28489	<.001	1.5918	3.6943
			day 4	1.5550*	.31208	.015	.4034	2.7066
			day 12	1.0600	.31208	.067	-.0916	2.2116
		day 12	day 0	1.5830*	.28489	.010	.5318	2.6343
			day 4	.4950	.31208	.461	-.6566	1.6466
			day 8	-1.0600	.31208	.067	-2.2116	.0916
W	Tukey HSD	day 0	day 4	-.0767	.32371	.995	-1.1133	.9600
			day 8	-.3700	.32371	.676	-1.4066	.6666
			day 12	-1.7700*	.32371	.003	-2.8066	-.7334
		day 4	day 0	.0767	.32371	.995	-.9600	1.1133
			day 8	-.2933	.32371	.802	-1.3300	.7433
			day 12	-1.6933*	.32371	.004	-2.7300	-.6567
		day 8	day 0	.3700	.32371	.676	-.6666	1.4066
			day 4	.2933	.32371	.802	-.7433	1.3300
			day 12	-1.4000*	.32371	.011	-2.4366	-.3634
		day 12	day 0	1.7700*	.32371	.003	.7334	2.8066
			day 4	1.6933*	.32371	.004	.6567	2.7300
			day 8	1.4000*	.32371	.011	.3634	2.4366
P	Tukey HSD	day 0	day 4	-.5633	.44832	.615	-2.0473	.9207
			day 8	-.7067	.40099	.363	-2.0340	.6207
			day 12	-.4033	.40099	.751	-1.7307	.9240
		day 4	day 0	.5633	.44832	.615	-.9207	2.0473
			day 8	-.1433	.44832	.988	-1.6273	1.3407
			day 12	.1600	.44832	.983	-1.3240	1.6440
		day 8	day 0	.7067	.40099	.363	-.6207	2.0340
			day 4	.1433	.44832	.988	-1.3407	1.6273
			day 12	.3033	.40099	.871	-1.0240	1.6307
		day 12	day 0	.4033	.40099	.751	-.9240	1.7307
			day 4	-.1600	.44832	.983	-1.6440	1.3240
			day 8	-.3033	.40099	.871	-1.6307	1.0240
CW	Tukey HSD	day 0	day 4	-.6100	.22390	.108	-1.3511	.1311
			day 8	-.5567	.22390	.147	-1.2978	.1845
			day 12	-1.8217*	.25033	<.001	-2.6503	-.9930
		day 4	day 0	.6100	.22390	.108	-.1311	1.3511
			day 8	.0533	.22390	.995	-.6878	.7945
			day 12	-1.2117*	.25033	.008	-2.0403	-.3830
		day 8	day 0	.5567	.22390	.147	-.1845	1.2978
			day 4	-.0533	.22390	.995	-.7945	.6878
			day 12	-1.2650*	.25033	.006	-2.0936	-.4364
		day 12	day 0	1.8217*	.25033	<.001	.9930	2.6503
			day 4	1.2117*	.25033	.008	.3830	2.0403
			day 8	1.2650*	.25033	.006	.4364	2.0936
CP	Tukey HSD	day 0	day 4	.1100	.44188	.994	-1.3527	1.5727
			day 8	-.3533	.44188	.853	-1.8160	1.1093
			day 12	.0467	.44188	1.000	-1.4160	1.5093
		day 4	day 0	-.1100	.44188	.994	-1.5727	1.3527
			day 8	-.4633	.39523	.661	-1.7716	.8449
			day 12	-.0633	.39523	.998	-1.3716	1.2449
		day 8	day 0	.3533	.44188	.853	-1.1093	1.8160
			day 4	.4633	.39523	.661	-.8449	1.7716
			day 12	.4000	.39523	.748	-.9083	1.7083
		day 12	day 0	-.0467	.44188	1.000	-1.5093	1.4160
			day 4	.0633	.39523	.998	-1.2449	1.3716
			day 8	-.4000	.39523	.748	-1.7083	.9083

Based on observed means.
The error term is Mean Square(Error) = .234.

*. The mean difference is significant at the 0.05 level.

APPENDIX C

Two-way ANOVA and Tukey's test of PPO activity, TPC and browning index between treatments

Table C.1: Two-way ANOVA of PPO activity.

Tests of Between-Subjects Effects

Dependent Variable: PPO

Storage	Source	Type III Sum of Squares	df	Mean Square	F	Sig.
day 0	Corrected Model	307448.970[a]	4	76862.242	268.958	<.001
	Intercept	347657.333	1	347657.333	1216.530	<.001
	Treatment	307448.970	4	76862.242	268.958	<.001
	Error	1714.667	6	285.778		
	Total	656976.000	11			
	Corrected Total	309163.636	10			
day 4	Corrected Model	98458.667[b]	4	24614.667	384.604	<.001
	Intercept	69481.846	1	69481.846	1085.654	<.001
	Treatment	98458.667	4	24614.667	384.604	<.001
	Error	448.000	7	64.000		
	Total	149088.000	12			
	Corrected Total	98906.667	11			
day 8	Corrected Model	159868.000[c]	4	39967.000	333.058	<.001
	Intercept	83758.154	1	83758.154	697.985	<.001
	Treatment	159868.000	4	39967.000	333.058	<.001
	Error	840.000	7	120.000		
	Total	221200.000	12			
	Corrected Total	160708.000	11			
day 12	Corrected Model	103513.333[d]	4	25878.333	606.523	<.001
	Intercept	51693.128	1	51693.128	1211.558	<.001
	Treatment	103513.333	4	25878.333	606.523	<.001
	Error	298.667	7	42.667		
	Total	171312.000	12			
	Corrected Total	103812.000	11			

a. R Squared = .994 (Adjusted R Squared = .991)

b. R Squared = .995 (Adjusted R Squared = .993)

c. R Squared = .995 (Adjusted R Squared = .992)

d. R Squared = .997 (Adjusted R Squared = .995)

Table C.2: Tukey's test of PPO activity.

Multiple Comparisons

Dependent Variable: PPO

Storage		(I) Heat trt	(J) Heat trt	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
day 0	Tukey HSD	Control	W	365.3333*	15.43205	<.001	307.4458	423.2209
			P	462.0000*	16.90496	<.001	398.5874	525.4126
			CW	392.0000*	16.90496	<.001	328.5874	455.4126
			CP	480.0000*	16.90496	<.001	416.5874	543.4126
		W	Control	-365.3333*	15.43205	<.001	-423.2209	-307.4458
			P	96.6667*	15.43205	.004	38.7791	154.5542
			CW	26.6667	15.43205	.483	-31.2209	84.5542
			CP	114.6667*	15.43205	.002	56.7791	172.5542
		P	Control	-462.0000*	16.90496	<.001	-525.4126	-398.5874
			W	-96.6667*	15.43205	.004	-154.5542	-38.7791
			CW	-70.0000*	16.90496	.033	-133.4126	-6.5874
			CP	18.0000	16.90496	.818	-45.4126	81.4126
		CW	Control	-392.0000*	16.90496	<.001	-455.4126	-328.5874
			W	-26.6667	15.43205	.483	-84.5542	31.2209
			P	70.0000*	16.90496	.033	6.5874	133.4126
			CP	88.0000*	16.90496	.011	24.5874	151.4126
		CP	Control	-480.0000*	16.90496	<.001	-543.4126	-416.5874
			W	-114.6667*	15.43205	.002	-172.5542	-56.7791
			P	-18.0000	16.90496	.818	-81.4126	45.4126
			CW	-88.0000*	16.90496	.011	-151.4126	-24.5874
day 4	Tukey HSD	Control	W	212.0000*	8.00000	<.001	183.3759	240.6241
			P	252.0000*	7.30297	<.001	225.8699	278.1301
			CW	156.0000*	8.00000	<.001	127.3759	184.6241
			CP	252.0000*	7.30297	<.001	225.8699	278.1301
		W	Control	-212.0000*	8.00000	<.001	-240.6241	-183.3759
			P	40.0000*	7.30297	.006	13.8699	66.1301
			CW	-56.0000*	8.00000	.001	-84.6241	-27.3759
			CP	40.0000*	7.30297	.006	13.8699	66.1301
		P	Control	-252.0000*	7.30297	<.001	-278.1301	-225.8699
			W	-40.0000*	7.30297	.006	-66.1301	-13.8699
			CW	-96.0000*	7.30297	<.001	-122.1301	-69.8699
			CP	.0000	6.53197	1.000	-23.3715	23.3715
		CW	Control	-156.0000*	8.00000	<.001	-184.6241	-127.3759
			W	56.0000*	8.00000	.001	27.3759	84.6241
			P	96.0000*	7.30297	<.001	69.8699	122.1301
			CP	96.0000*	7.30297	<.001	69.8699	122.1301
		CP	Control	-252.0000*	7.30297	<.001	-278.1301	-225.8699
			W	-40.0000*	7.30297	.006	-66.1301	-13.8699
			P	.0000	6.53197	1.000	-23.3715	23.3715
			CW	-96.0000*	7.30297	<.001	-122.1301	-69.8699

Table C.2 (continued): Tukey's test of PPO activity.

day 8	Tukey HSD	Control	W	274.0000*	10.95445	<.001	234.8048	313.1952
			P	324.0000*	10.00000	<.001	288.2199	359.7801
			CW	272.0000*	10.95445	<.001	232.8048	311.1952
			CP	324.0000*	10.00000	<.001	288.2199	359.7801
		W	Control	-274.0000*	10.95445	<.001	-313.1952	-234.8048
			P	50.0000*	10.00000	.010	14.2199	85.7801
			CW	-2.0000	10.95445	1.000	-41.1952	37.1952
			CP	50.0000*	10.00000	.010	14.2199	85.7801
		P	Control	-324.0000*	10.00000	<.001	-359.7801	-288.2199
			W	-50.0000*	10.00000	.010	-85.7801	-14.2199
			CW	-52.0000*	10.00000	.008	-87.7801	-16.2199
			CP	.0000	8.94427	1.000	-32.0027	32.0027
		CW	Control	-272.0000*	10.95445	<.001	-311.1952	-232.8048
			W	2.0000	10.95445	1.000	-37.1952	41.1952
			P	52.0000*	10.00000	.008	16.2199	87.7801
			CP	52.0000*	10.00000	.008	16.2199	87.7801
		CP	Control	-324.0000*	10.00000	<.001	-359.7801	-288.2199
			W	-50.0000*	10.00000	.010	-85.7801	-14.2199
			P	.0000	8.94427	1.000	-32.0027	32.0027
			CW	-52.0000*	10.00000	.008	-87.7801	-16.2199
day 12	Tukey HSD	Control	W	166.6667*	5.96285	<.001	145.3315	188.0018
			P	230.6667*	5.33333	<.001	211.5839	249.7494
			CW	190.6667*	5.96285	<.001	169.3315	212.0018
			CP	230.6667*	5.96285	<.001	209.3315	252.0018
		W	Control	-166.6667*	5.96285	<.001	-188.0018	-145.3315
			P	64.0000*	5.96285	<.001	42.6649	85.3351
			CW	24.0000*	6.53197	.044	.6285	47.3715
			CP	64.0000*	6.53197	<.001	40.6285	87.3715
		P	Control	-230.6667*	5.33333	<.001	-249.7494	-211.5839
			W	-64.0000*	5.96285	<.001	-85.3351	-42.6649
			CW	-40.0000*	5.96285	.002	-61.3351	-18.6649
			CP	.0000	5.96285	1.000	-21.3351	21.3351
		CW	Control	-190.6667*	5.96285	<.001	-212.0018	-169.3315
			W	-24.0000*	6.53197	.044	-47.3715	-.6285
			P	40.0000*	5.96285	.002	18.6649	61.3351
			CP	40.0000*	6.53197	.003	16.6285	63.3715
		CP	Control	-230.6667*	5.96285	<.001	-252.0018	-209.3315
			W	-64.0000*	6.53197	<.001	-87.3715	-40.6285
			P	.0000	5.96285	1.000	-21.3351	21.3351
			CW	-40.0000*	6.53197	.003	-63.3715	-16.6285

Based on observed means.
The error term is Mean Square(Error) = 42.667.

*. The mean difference is significant at the 0.05 level.

Table C.3: Two-way ANOVA of TPC results.

Tests of Between-Subjects Effects

Dependent Variable: TPC

Storage	Source	Type III Sum of Squares	df	Mean Square	F	Sig.
day 0	Corrected Model	561.136[a]	4	140.284	36.397	<.001
	Intercept	29312.136	1	29312.136	7605.168	<.001
	Treatment	561.136	4	140.284	36.397	<.001
	Error	26.980	7	3.854		
	Total	31844.909	12			
	Corrected Total	588.115	11			
day 4	Corrected Model	1478.023[b]	4	369.506	83.209	<.001
	Intercept	41861.647	1	41861.647	9426.858	<.001
	Treatment	1478.023	4	369.506	83.209	<.001
	Error	44.407	10	4.441		
	Total	43384.076	15			
	Corrected Total	1522.429	14			
day 8	Corrected Model	2224.009[c]	4	556.002	344.809	<.001
	Intercept	28500.236	1	28500.236	17674.615	<.001
	Treatment	2224.009	4	556.002	344.809	<.001
	Error	11.287	7	1.612		
	Total	33361.166	12			
	Corrected Total	2235.296	11			
day 12	Corrected Model	1158.848[d]	4	289.712	69.067	<.001
	Intercept	38069.299	1	38069.299	9075.627	<.001
	Treatment	1158.848	4	289.712	69.067	<.001
	Error	37.752	9	4.195		
	Total	39891.905	14			
	Corrected Total	1196.600	13			

a. R Squared = .954 (Adjusted R Squared = .928)

b. R Squared = .971 (Adjusted R Squared = .959)

c. R Squared = .995 (Adjusted R Squared = .992)

d. R Squared = .968 (Adjusted R Squared = .954)

Table C.4: Tukey's test of TPC results.

Multiple Comparisons

Dependent Variable: TPC

Storage		(I) Heat trt	(J) Heat trt	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
day 0	Tukey HSD	Control	W	11.01000*	1.963222	.005	3.98557	18.03443
			P	5.90600	1.963222	.102	-1.11843	12.93043
			CW	6.58650*	1.792168	.044	.17410	12.99890
			CP	-7.99617*	1.792168	.017	-14.40857	-1.58377
		W	Control	-11.01000*	1.963222	.005	-18.03443	-3.98557
			P	-5.10400	1.963222	.172	-12.12843	1.92043
			CW	-4.42350	1.792168	.202	-10.83590	1.98890
			CP	-19.00617*	1.792168	<.001	-25.41857	-12.59377
		P	Control	-5.90600	1.963222	.102	-12.93043	1.11843
			W	5.10400	1.963222	.172	-1.92043	12.12843
			CW	.68050	1.792168	.995	-5.73190	7.09290
			CP	-13.90217*	1.792168	<.001	-20.31457	-7.48977
		CW	Control	-6.58650*	1.792168	.044	-12.99890	-.17410
			W	4.42350	1.792168	.202	-1.98890	10.83590
			P	-.68050	1.792168	.995	-7.09290	5.73190
			CP	-14.58267*	1.602964	<.001	-20.31809	-8.84724
		CP	Control	7.99617*	1.792168	.017	1.58377	14.40857
			W	19.00617*	1.792168	<.001	12.59377	25.41857
			P	13.90217*	1.792168	<.001	7.48977	20.31457
			CW	14.58267*	1.602964	<.001	8.84724	20.31809
day 4	Tukey HSD	Control	W	27.02633*	1.720597	<.001	21.36371	32.68896
			P	23.01633*	1.720597	<.001	17.35371	28.67896
			CW	25.98133*	1.720597	<.001	20.31871	31.64396
			CP	17.28033*	1.720597	<.001	11.61771	22.94296
		W	Control	-27.02633*	1.720597	<.001	-32.68896	-21.36371
			P	-4.01000	1.720597	.212	-9.67262	1.65262
			CW	-1.04500	1.720597	.971	-6.70762	4.61762
			CP	-9.74600*	1.720597	.002	-15.40862	-4.08338
		P	Control	-23.01633*	1.720597	<.001	-28.67896	-17.35371
			W	4.01000	1.720597	.212	-1.65262	9.67262
			CW	2.96500	1.720597	.463	-2.69762	8.62762
			CP	-5.73600*	1.720597	.047	-11.39862	-.07338
		CW	Control	-25.98133*	1.720597	<.001	-31.64396	-20.31871
			W	1.04500	1.720597	.971	-4.61762	6.70762
			P	-2.96500	1.720597	.463	-8.62762	2.69762
			CP	-8.70100*	1.720597	.003	-14.36362	-3.03838
		CP	Control	-17.28033*	1.720597	<.001	-22.94296	-11.61771
			W	9.74600*	1.720597	.002	4.08338	15.40862
			P	5.73600*	1.720597	.047	.07338	11.39862
			CW	8.70100*	1.720597	.003	3.03838	14.36362

Table C.4 (continued): Tukey's test of TPC results.

day 8	Tukey HSD	Control	W	33.96317*	1.159201	<.001	29.81553	38.11080
			P	33.64733*	1.036821	<.001	29.93758	37.35709
			CW	27.32767*	1.159201	<.001	23.18003	31.47530
			CP	26.08867*	1.159201	<.001	21.94103	30.23630
		W	Control	-33.96317*	1.159201	<.001	-38.11080	-29.81553
			P	-.31583	1.159201	.998	-4.46347	3.83180
			CW	-6.63550*	1.269841	.007	-11.17901	-2.09199
			CP	-7.87450*	1.269841	.003	-12.41801	-3.33099
		P	Control	-33.64733*	1.036821	<.001	-37.35709	-29.93758
			W	.31583	1.159201	.998	-3.83180	4.46347
			CW	-6.31967*	1.159201	.006	-10.46730	-2.17203
			CP	-7.55867*	1.159201	.002	-11.70630	-3.41103
		CW	Control	-27.32767*	1.159201	<.001	-31.47530	-23.18003
			W	6.63550*	1.269841	.007	2.09199	11.17901
			P	6.31967*	1.159201	.006	2.17203	10.46730
			CP	-1.23900	1.269841	.858	-5.78251	3.30451
		CP	Control	-26.08867*	1.159201	<.001	-30.23630	-21.94103
			W	7.87450*	1.269841	.003	3.33099	12.41801
			P	7.55867*	1.159201	.002	3.41103	11.70630
			CW	1.23900	1.269841	.858	-3.30451	5.78251
day 12	Tukey HSD	Control	W	25.23767*	1.672259	<.001	19.61457	30.86077
			P	21.10567*	1.672259	<.001	15.48257	26.72877
			CW	10.03567*	1.869642	.003	3.74885	16.32249
			CP	12.25933*	1.672259	<.001	6.63623	17.88243
		W	Control	-25.23767*	1.672259	<.001	-30.86077	-19.61457
			P	-4.13200	1.672259	.181	-9.75510	1.49110
			CW	-15.20200*	1.869642	<.001	-21.48882	-8.91518
			CP	-12.97833*	1.672259	<.001	-18.60143	-7.35523
		P	Control	-21.10567*	1.672259	<.001	-26.72877	-15.48257
			W	4.13200	1.672259	.181	-1.49110	9.75510
			CW	-11.07000*	1.869642	.002	-17.35682	-4.78318
			CP	-8.84633*	1.672259	.003	-14.46943	-3.22323
		CW	Control	-10.03567*	1.869642	.003	-16.32249	-3.74885
			W	15.20200*	1.869642	<.001	8.91518	21.48882
			P	11.07000*	1.869642	.002	4.78318	17.35682
			CP	2.22367	1.869642	.757	-4.06315	8.51049
		CP	Control	-12.25933*	1.672259	<.001	-17.88243	-6.63623
			W	12.97833*	1.672259	<.001	7.35523	18.60143
			P	8.84633*	1.672259	.003	3.22323	14.46943
			CW	-2.22367	1.869642	.757	-8.51049	4.06315

Based on observed means.
The error term is Mean Square(Error) = 3.566.

*. The mean difference is significant at the 0.05 level.

Table C.5: Two-way ANOVA of browning index results.

Tests of Between-Subjects Effects

Dependent Variable: Browning index

Storage	Source	Type III Sum of Squares	df	Mean Square	F	Sig.
day 0	Corrected Model	2.239[a]	4	.560	4.414	.030
	Intercept	286.243	1	286.243	2257.397	<.001
	Treatment	2.239	4	.560	4.414	.030
	Error	1.141	9	.127		
	Total	305.297	14			
	Corrected Total	3.380	13			
day 4	Corrected Model	2.806[b]	4	.701	4.085	.043
	Intercept	213.831	1	213.831	1245.256	<.001
	Treatment	2.806	4	.701	4.085	.043
	Error	1.374	8	.172		
	Total	230.152	13			
	Corrected Total	4.179	12			
day 8	Corrected Model	9.770[c]	4	2.442	18.814	<.001
	Intercept	182.235	1	182.235	1403.747	<.001
	Treatment	9.770	4	2.442	18.814	<.001
	Error	1.168	9	.130		
	Total	210.296	14			
	Corrected Total	10.938	13			
day 12	Corrected Model	4.160[d]	4	1.040	4.342	.037
	Intercept	150.974	1	150.974	630.384	<.001
	Treatment	4.160	4	1.040	4.342	.037
	Error	1.916	8	.239		
	Total	170.050	13			
	Corrected Total	6.076	12			

a. R Squared = .662 (Adjusted R Squared = .512)

b. R Squared = .671 (Adjusted R Squared = .507)

c. R Squared = .893 (Adjusted R Squared = .846)

d. R Squared = .685 (Adjusted R Squared = .527)

Table C.6: Tukey's test of browning index results.

Multiple Comparisons

Dependent Variable: Browning index

Storage		(I) Heat trt	(J) Heat trt	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
day 0	Tukey HSD	Control	W	.3420	.29075	.764	-.6357	1.3196
			P	.1953	.29075	.958	-.7824	1.1730
			CW	.0686	.29075	.999	-.9090	1.0463
			CP	-.9380	.32507	.100	-2.0311	.1550
		W	Control	-.3420	.29075	.764	-1.3196	.6357
			P	-.1467	.29075	.985	-1.1243	.8310
			CW	-.2733	.29075	.874	-1.2510	.7043
			CP	-1.2800*	.32507	.022	-2.3731	-.1869
		P	Control	-.1953	.29075	.958	-1.1730	.7824
			W	.1467	.29075	.985	-.8310	1.1243
			CW	-.1267	.29075	.991	-1.1043	.8510
			CP	-1.1333*	.32507	.042	-2.2264	-.0403
		CW	Control	-.0686	.29075	.999	-1.0463	.9090
			W	.2733	.29075	.874	-.7043	1.2510
			P	.1267	.29075	.991	-.8510	1.1043
			CP	-1.0067	.32507	.074	-2.0997	.0864
		CP	Control	.9380	.32507	.100	-.1550	2.0311
			W	1.2800*	.32507	.022	.1869	2.3731
			P	1.1333*	.32507	.042	.0403	2.2264
			CW	1.0067	.32507	.074	-.0864	2.0997
day 4	Tukey HSD	Control	W	1.3533*	.37828	.042	.0465	2.6602
			P	.7200	.41439	.465	-.7116	2.1516
			CW	.5467	.37828	.619	-.7602	1.8535
			CP	.2600	.37828	.954	-1.0469	1.5669
		W	Control	-1.3533*	.37828	.042	-2.6602	-.0465
			P	-.6333	.37828	.497	-1.9402	.6735
			CW	-.8067	.33835	.213	-1.9756	.3622
			CP	-1.0933	.33835	.068	-2.2622	.0756
		P	Control	-.7200	.41439	.465	-2.1516	.7116
			W	.6333	.37828	.497	-.6735	1.9402
			CW	-.1733	.37828	.989	-1.4802	1.1335
			CP	-.4600	.37828	.744	-1.7669	.8469
		CW	Control	-.5467	.37828	.619	-1.8535	.7602
			W	.8067	.33835	.213	-.3622	1.9756
			P	.1733	.37828	.989	-1.1335	1.4802
			CP	-.2867	.33835	.908	-1.4556	.8822
		CP	Control	-.2600	.37828	.954	-1.5669	1.0469
			W	1.0933	.33835	.068	-.0756	2.2622
			P	.4600	.37828	.744	-.8469	1.7669
			CW	.2867	.33835	.908	-.8822	1.4556

Table C.6 (continued): Tukey's test of browning index results.

day 8	Tukey HSD	Control	W	2.6150*	.32891	<.001	1.5090	3.7210
			P	2.1317*	.32891	<.001	1.0257	3.2377
			CW	2.1550*	.32891	<.001	1.0490	3.2610
			CP	1.3517*	.32891	.017	.2457	2.4577
		W	Control	-2.6150*	.32891	<.001	-3.7210	-1.5090
			P	-.4833	.29419	.509	-1.4726	.5059
			CW	-.4600	.29419	.552	-1.4492	.5292
			CP	-1.2633*	.29419	.013	-2.2526	-.2741
		P	Control	-2.1317*	.32891	<.001	-3.2377	-1.0257
			W	.4833	.29419	.509	-.5059	1.4726
			CW	.0233	.29419	1.000	-.9659	1.0126
			CP	-.7800	.29419	.140	-1.7692	.2092
		CW	Control	-2.1550*	.32891	<.001	-3.2610	-1.0490
			W	.4600	.29419	.552	-.5292	1.4492
			P	-.0233	.29419	1.000	-1.0126	.9659
			CP	-.8033	.29419	.125	-1.7926	.1859
		CP	Control	-1.3517*	.32891	.017	-2.4577	-.2457
			W	1.2633*	.29419	.013	.2741	2.2526
			P	.7800	.29419	.140	-.2092	1.7692
			CW	.8033	.29419	.125	-.1859	1.7926
day 12	Tukey HSD	Control	W	.1550	.44674	.996	-1.3884	1.6984
			P	1.3750	.44674	.084	-.1684	2.9184
			CW	-.1700	.48938	.996	-1.8607	1.5207
			CP	.6917	.44674	.563	-.8517	2.2351
		W	Control	-.1550	.44674	.996	-1.6984	1.3884
			P	1.2200	.39958	.087	-.1604	2.6004
			CW	-.3250	.44674	.944	-1.8684	1.2184
			CP	.5367	.39958	.675	-.8438	1.9171
		P	Control	-1.3750	.44674	.084	-2.9184	.1684
			W	-1.2200	.39958	.087	-2.6004	.1604
			CW	-1.5450*	.44674	.050	-3.0884	-.0016
			CP	-.6833	.39958	.478	-2.0638	.6971
		CW	Control	.1700	.48938	.996	-1.5207	1.8607
			W	.3250	.44674	.944	-1.2184	1.8684
			P	1.5450*	.44674	.050	.0016	3.0884
			CP	.8617	.44674	.375	-.6817	2.4051
		CP	Control	-.6917	.44674	.563	-2.2351	.8517
			W	-.5367	.39958	.675	-1.9171	.8438
			P	.6833	.39958	.478	-.6971	2.0638
			CW	-.8617	.44674	.375	-2.4051	.6817

Based on observed means.
The error term is Mean Square(Error) = .239.

*. The mean difference is significant at the 0.05 level.

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FACULTY OF Science

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ID Number(s)	22ADB05521
Programme / Course	Bachelor of Science (Honours) Food Science
Title of Final Year Project	Study on Interactive Effect of Heat Treatment and Enzymatic Activities in Mature Coconut Water Upon Storage

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Date: 07/10/2025

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