EFFECT OF COOKING METHODS AND COOKING OILS ON COLOR, TEXTURE AND ANTIOXIDATIVE PROPERTIES OF BROWN RICE

By

CHEN JIA LING

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ABSTRACT

EFFECT OF COOKING METHODS AND COOKING OILS ON COLOR, TEXTURE AND ANTIOXIDATIVE PROPERTIES OF BROWN RICE

CHEN JIA LING

Brown rice consumption is in continuing trends due to its health-promoting features. Effects of cooking on physical and antioxidative properties of brown rice were investigated by applying three different cooking methods involving steaming (40 mins) and/or frying (1 min) with/without palm and coconut oils. For color measurement, lightness (L*) of control was significantly decreased in cooking methods I and II. Addition of palm and coconut oils did not significantly affect a* and b* of treated rice in all cooking methods. With the control as a reference, there was an insignificant ($p > 0.05$) difference between total color differences of oil-treated rice. Furthermore, results of texture analysis reported a significant ($p < 0.05$) decrease in hardness and increase in stickiness of control in cooking methods I and II. However, in cooking methods I and II, oil addition significantly increased hardness and decreased stickiness of treated rice. For determination of antioxidative properties, total phenolic contents (TPC) and total flavonoid contents (TFC) of cooked brown rice were evaluated. Results of TPC and TFC of cooked rice that were respectively examined by Folin-Ciocalteau's test and aluminium chloride colorimetric methods demonstrated that cooking methods I and II significantly reduced antioxidant contents of control. Nevertheless, in cooking methods I and II, palm oil treated

rice had significantly higher TPC and TFC than coconut oil treated rice and control. Lastly, antioxidant activity was studied by DPPH and reducing power assays and interpreted as IC_{50} and EC_{50}, respectively. Inverse proportion between TPC and TFC with IC_{50} and EC_{50} values corroborated the lower IC_{50} and EC_{50} values of palm oil treated rice with higher antioxidant activity in cooking methods I and II. Therefore, treating brown rice with palm oil using cooking methods I and II was recommended, due to the firmer texture, and its high antioxidant contents and activities.

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DECLARATION

I hereby declare that this final year project report entitled "**EFFECT OF COOKING METHODS AND COOKING OILS ON COLOR, TEXTURE AND ANTIOXIDATIVE PROPERTIES OF BROWN RICE**" 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.

CHEN JIA LING

APPROVAL SHEET

This final year project report entitled "**EFFECT OF COOKING METHODS AND COOKING OILS ON COLOR, TEXTURE AND ANTIOXIDATIVE PROPERTIES OF BROWN RICE**" was prepared by CHEN JIA LING and submitted as partial fulfilment of the requirements for the degree of Bachelor of Science (Honors) Food Science at Universiti Tunku Abdul Rahman.

Approved by:

Lye Huey Shi

_____

Dr. Lye Huey Shi Date: …13 MAY 2023…….

Supervisor

Department of Agricultural and Food Science

Faculty of Science

Universiti Tunku Abdul Rahman

_____

Dr. Loo Keat Wei Date: …13 MAY 2023…….

Co-Supervisor

Department of Biological Science

Faculty of Science

Universiti Tunku Abdul Rahman

FACULTY OF SCIENCE

UNIVERSITI TUNKU ABDUL RAHMAN

Date: _13th MAY 2023____

PERMISSION SHEET

It is hereby certified that **CHEN JIA LING** (ID No: **19ADB03363**) has completed this final year project report entitled "**EFFECT OF COOKING METHODS AND COOKING OILS ON COLOR, TEXTURE AND ANTIOXIDATIVE PROPERTIES OF BROWN RICE**" under the supervision of Dr. Lye Huey Shi (Supervisor) from the Department of Agricultural and Food Science, Faculty of Science, and Dr. Loo Keat Wei (Co-Supervisor) from the Department of Biological Science, Faculty of Science.

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

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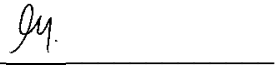

CHEN JIA LING

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FACULTY OF ___SCIENCE_____

Date: 13th MAY 2023

SUBMISSION OF FINAL YEAR PROJECT

I, **CHEN JIA LING** (ID No: **19ADB03363**) hereby certify that I have completed the final year project titled "**EFFECT OF COOKING METHODS AND COOKING OILS ON COLOR, TEXTURE AND ANTIOXIDATIVE PROPERTIES OF BROWN RICE**" under the supervision of Dr. Lye Huey Shi (Supervisor) from the Department of Agricultural and Food Science, Faculty of Science, and Dr. Loo Keat Wei (Co-Supervisor) from the Department of Biological Science, Faculty of Science.

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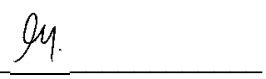

CHEN JIA LING

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ΔE*	Total color difference
α-carotene	Alpha-carotene
α-tocopherol	Alpha-tocopherol
α-tocotrienol	Alpha-tocotrienol
β-carotene dioxygenase	Beta-carotene dioxygenase
β-glucan	Beta-glucan
δ-tocopherol	Delta-tocopherol
δ-tocotrienol	Delta-tocotrienol
γ-carotene	Gamma-carotene
γ-tocopherol	Gamma-tocopherol
γ-tocotrienol	Gamma-tocotrienol
a*	Redness-greenness
$ABTS^{\bullet+}$	2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) cation
Al^{3+}	Aluminum ion
$AlCl_3$	Aluminum chloride
ANOVA	Analysis of variance
b*	Yellowness-blueness
C	Carbon
$C_2HCl_3O_2$	Trichloroacetic acid
CGE	Cyanidin-3-*O*-glucoside equivalent
CIE	Commission Internationale de I'Elcairage
Cu^{+}	Copper (I) ion

DPPH	2,2-diphenyl-1-picryl-hydrazyl
DW	Dry weight
EC_{50}	Half-maximal effective concentration
F/B	Firmicutes/Bacteroidetes ratio
FC	Folin-Ciocalteu
Fe^{2+}	Ferrous ion
Fe^{3+}	Ferric ion
$FeCl_3 \cdot 6H_2O$	Ferric chloride hexahydrate
GAE	Gallic acid equivalent
H^+	Hydrogen ion
$H_3PMo_{12}O_{40}$	Phosphomolybdic acid
H_3PO_4	Phosphoric acid
$H_3PW_{12}O_{40}$	Phosphotungstic acid
H_6NaO_6P	Sodium phosphate monobasic dihydrate
$H_{25}Na_2O_{16}P$	Sodium phosphate dibasic dodecahydrate
HAT	Hydrogen atom transfer
HbA1c	Hemoglobin A1c
HPLC	High performance liquid chromatography
IC_{50}	Half-maximal inhibitory concentration
K_3 [Fe (CN_6)]	Potassium ferricyanide
L*	Lightness-darkness
LDL	Low density lipoprotein
Mo	Molybdenum
Na_2CO_3	Sodium carbonate
$NaNO_2$	Sodium nitrite

NaOH	Sodium hydroxide
QUE	Quercetin equivalent
RDI	Recommended daily intake
RE	Rutin equivalent
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
rpm	Revolution per minute
SCFAs	Short-chain fatty acids
SD	Standard deviation
SOD	Superoxide dismutase
SPSS	Statistical Package for the Social Science
TFC	Total flavonoid content
TPA	Texture profile analysis
TPC	Total phenolic content
Tukey’s HSD	Tukey’s honestly significant difference
UV-Vis	Ultraviolet-visible
v/v	Volume per volume
w/v	Weight per volume

CHAPTER 1

INTRODUCTION

Rice is a common staple food in daily diet, especially of Asians. Rice genotypes, either non-pigmented (white) or pigmented such as black, red, and brown rice are determined by the absence or presence and the types of pigments found in rice grains. For instance, in pericarp and nucellar layers of grains (Fracassetti, et al., 2020). In this context, reddish-brown color of brown rice is attributed to the presence of pigments such as carotenoids and anthocyanins in the bran layers of the rice kernels (Noh and Zik, 2002). Besides, other sensory attributes such as rice texture also influence its eating quality. Brown rice with intact bran layers tends to be chewier (Wang, et al., 2011). Hence, consumer who is not preferring the harder texture of brown rice may shift to consuming other types of rice with which white rice is most commonly consumed.

Despite the undesirable texture of cooked brown rice, brown rice is valuable in terms of its antioxidative properties with the presence of bioactive compounds in its bran fractions. Phytochemicals such as phenolics, flavonoids, γ-oryzanols and proanthocyanins are natural antioxidants which render brown rice its antioxidative properties in minimizing risks of diseases related to oxidative stress (Saikia, et al., 2012; Wu, et al., 2018). Nutritional profiles of brown rice are improved by its contents of minerals and vitamins which are at least double that of other types of rice due to its retention of bran layers (Deepa, Singh and Naidu, 2008).

According to Garber, et al. (2013), bran layers may decrease the rate of water absorption during rice cooking due to their roles as water barrier. Therefore, as a comparison between brown rice and other varieties of rice, longer cooking time is necessary for the brown rice. The common methods used to cook rice include using rice cooker or pressure cooker, boiling or wet cooking in boiling water, also known as steaming which is considered as the hydrothermal process (Min, McClung and Chen, 2014). Despite the improved bioavailability of nutrients in the cooked brown rice due to the elimination of antinutritional factors during thermal treatments, cooking may negatively affect the contents and activity of natural antioxidants of brown rice (Ma, et al., 2005). Hence, while cooking brown rice which is rich in the bioactive substances, duration and temperature of cooking must be well-controlled.

Oil palm tree with species of *Elaeis guineensis* is the widespread origin of palm oil (Boateng, et al., 2016). Being the main producer and export of palm oil, palm oil is renowned for its importance as the economical backbone of Malaysia. Other edible vegetable oil such as coconut oil with origin of *Cocos nucifera* is also produced in Malaysia as the top five industrial crop (Assuncao, et al., 2008). Palm and coconut oils are rich in saturated fatty acids, with palmitic and lauric acids being the predominant saturated fatty acids in the oils, respectively (Mancini, et al., 2015). Major antioxidants present in these types of cooking oils are vitamin E (tocopherol and tocotrienol) and carotenoids (Kumar and Krishna, 2015; Tan, et al., 2021). Hence, antioxidative potentials of the cooking oils such as anti-inflammatory properties have been well-documented (Tan, et al., 2021).

Due to increasing health awareness of consumers for nutrient-rich foods, nutrient-dense brown rice with medium glycemic index marks its potential to be further cropped up with functional properties and desired physicochemical characteristics. However, most of the studies have been focused on proximate analysis of brown rice. There is scanty literature studying the effect of cooking on brown rice (Fracassetti, et al., 2020). Therefore, a study emphasizing the physical, sensory and antioxidative properties of cooked brown rice was conducted. This was to provide an insight for food manufacturers to develop brown rice based processed products using optimum processing conditions which preserved the sensory attributes and minimized the loss of bioactive compounds.

The objectives of this study were (1) to examine the effect of different cooking methods and cooking oils (palm and coconut oils) on color and texture of brown rice, (2) to determine total phenolic contents and total flavonoid contents of brown rice cooked by different cooking methods and/or cooking oils, and (3) to evaluate the antioxidative properties of brown rice cooked by different cooking methods and/or cooking oils through 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free radical scavenging and reducing power assays.

CHAPTER 2

LITERATURE REVIEW

2.1 Brown Rice

2.1.1 Processing

Brown rice is also termed as the least processed rice which only involves de-husking after the pre-treatments such as cleaning, as compared to white rice that requires the process of milling or polishing. Precleaning of paddy for the removal of undesirable contaminants such as soil and straw is required prior to dehulling to ensure the efficient operation of the de-husking machine. The impurities can be separated from the paddy according to their weight, size, and density using an aspirator (Nambi, Manickavasagan and Sultan, 2017).

Rubber roll sheller is commonly used in dehulling in the commercial scale due to its higher dehulling efficiency of up to 90% in one run and hence, higher brown rice recovery as compared to centrifugal sheller and disc sheller. In rubber roll sheller, difference in the rotation speeds of two abrasively surfaced rubber rollers where the paddy grains are passed through creates a frictional force to get rid of the hulls from the paddy. The process is followed by the removal of hull by suction and being disposed into the storage dump (Ferranti, Berry and Anderson, 2019). According to the study performed by Firouzi, Alizadeh and Minaei (2010), the husking ratio during dehulling was found to be significantly affected by the differential speeds of the two rollers. An increasing

trend of husking index from 55% to 60% was shown with the increasing differential speeds from 1.5 m/s to 2.9 m/s at the constant moisture content of paddy grains of 8.5%. At slower roller speed, husks were not removed from the paddy grains due to lower strength of shearing forces of the two rollers. This led to a smaller husking index which indicated a lower proportion of de-husked brown rice. Therefore, the rotational speed of the two rollers in the rubber roll sheller was recommended to be fixed at 2.9 m/s to maximize the percentage of dehulled brown rice (Payman, et al., 2007).

After dehulling process, the de-husked brown rice may be mixed with 10% of unhusked paddy after the dehulling process (Ferranti, Berry and Anderson, 2019). This raises the need for the separation of the unwanted paddy grains with husks from the brown rice using the sieving-cum aspiration based on their size, buoyancy, and specific gravity (Nambi, Manickavasagan and Sultan, 2017).

2.1.2 Physical Characteristics

Physical properties of brown rice must be investigated for quality control and a proper design of the machines used in postharvest handling and processing of paddy grains. Pertaining to the study on brown rice conducted by Tiwari, Dayma and Sharma (2017), the mean length, width and thickness of 100 long grain brown rice kernels were 6.393 mm, 2.14 mm and 1.9 mm, respectively. It is necessary to identify the size and shape of brown rice kernels to design a machine which fits the purpose of sifting unhusked brown rice out from other undesirables. The authors reported that the size which was calculated as the

product of length, width and thickness was around 25.994 mm^3. Brown rice was not in spherical shape as the sphericity of brown rice was found to be 42.68% which was below 70%. Thousand grains weight of 24.23 g was associated with the grading of grain size and maturity of brown rice kernels (Liu, et al., 2009). On the other hand, the bulk density of brown rice kernels serves as a source of information of the necessary storage space, transportation, and packaging requirements. In this context, it was shown that brown rice kernels had an average bulk density of 553.18 kg/m^3. In terms of true density, a mean true density of brown rice kernels was 1251.44 kg/m^3, which was higher than that of water to ease the separation and cleaning processes (Varnamkhasti, et al., 2007). The porosity of brown rice kernels measured was within the standard value of 55.7% which was an important parameter of storage quality and texture of brown rice (Kocabiyik, Aktas and Kayisoglu, 2004).

In the view of physical structure, brown rice kernel consists of bran, germ, and endosperm. Before removing the husks of paddy grains, husks make up 20% of the grain weight. In the unhusked kernels, bran layers which act as the outer protective layers of kernels are composed of 1%–2% of pericarp and 5% of aleurone layers which are responsible for the enclosure of germ (1%) located in the starchy endosperms which occupy around 90% of a brown rice kernel (Ferranti, Berry and Anderson, 2019).

2.1.3 Sensory Attributes

The presence of carotenoids such as lycopene and carotene in the bran layers of brown rice kernels contributes to the pigmentation of brown rice with their reddish-brown or tan color (Oli, et al., 2016). With the bran layers that are not being removed from the paddy grains by milling, fibrous bran layers give rise to a chewy texture of the cooked brown rice (Wang, et al., 2011). Adi and his co-workers (2020) also showed that the texture of brown rice may also be influenced by the amylose (degree of polymerization: 100–20000) and amylopectin contents, in which brown rice with high amylose content of around 20.5% was firmer and less fluffy in texture than the white rice with amylose content of less than 20%. This could be due to the negative correlation between the amylose and amylopectin contents with the proportion of open structure of starch granules that affected the degree of starch hydration (Kibar and Ferhunde, 2010). With regard to this, there was a significant difference ($p < 0.05$) in the overall liking of the texture (hardness, chewiness and stickiness) of brown rice and white rice. A higher consumers' acceptance to the texture of white rice over brown rice was demonstrated in the 9-point hedonic scaling test involving 140 trained sensory panelists (Gondal, et al., 2021). The flavor and aroma of uncooked brown rice are in a nutty flavor while the foods of cooked brown rice are found to be scented like popcorn or buttery flavor due to the presence of aromatic compounds such as furfural and 2-acetyl-1-pyroline, respectively (Garber, 2013; Lina and Min, 2022). However, there is limited research justifying the concentration of the aromatic compounds required to impart the flavor and aroma of brown rice, both in the cooked and uncooked forms.

2.1.4 Nutritional Compositions

Proximate analysis of brown rice is required for the labelling purposes as part of the legal requirements to provide information to consumers about the nutritional values of brown rice products while making purchasing choice. Proximate analysis emphasizes on the analysis of macronutrient contents such as water, carbohydrates, lipids, proteins, and dietary fibers. To be more comprehensive, micronutrients such as vitamins and minerals are also included (Ganogpichayagrai and Suksaard, 2020).

Unhusked brown rice kernels are categorized as shelf-stable foods with low moisture contents of around 14% that are not hospitable for microbial growth during storage at room temperature (Kumar, et al., 2016). Macronutrients in addition to the dietary fibers are mostly found in the bran layers of brown rice kernels. For instance, a nutritional composition that is made up of an average of 17% lipids, 14% proteins, 25% dietary fibers such as cellulose and lignin, as well as 8.5% of ashes in the bran layers (Ferranti, Berry and Anderson, 2019).

In terms of micronutrients, most of the vitamins and minerals are present in the germ of brown rice kernels. This can be seen in the contents such as B vitamins, vitamin E, manganese, selenium, calcium, magnesium, and phosphorus (LaMacro, 2019). Due to the retention of nutrient-dense bran, germ and endosperm, brown rice is also considered as wholegrain (Laseter, 2019). As compared to white rice with its bran and germ being removed, a study proposed by LaMacro (2019) showed that brown rice was 3, 4 and 10 times richer in the

respective contents of niacin, thiamine, and pantothenic acids which contributed to 15%, 12% and 14% of the recommended daily intake (RDI), respectively.

2.1.5 Potential Health Benefits

Controlling Diabetes

The suitability of foods to be consumed by type II diabetic patients is governed by glycemic index of the food or the effect of food consumption on post-meal blood glucose level. By comparing the glycemic index of brown rice and white rice, brown rice records a glycemic index of 50 which is lower than white rice with glycemic index of 72 (Trinidad, et al., 2012). Medium glycemic index of brown rice is mainly due to the presence of complex carbohydrates which help in controlling the blood glucose level. Complex carbohydrates such as fibers are resistant to digestion to prevent breakdown into simple sugars that may affect the post-meal blood sugar level (Fuiji, et al., 2013). Moreover, fibers can be fermented by microflora in colon to synthesize short chain fatty acids (SCFAs) such as butyrate and propionate. These SCFAs improve the metabolization of glucose and insulin sensitivity (Canfora, Jocken and Blaak, 2015). In addition to the high contents of dietary fibers, magnesium contents in brown rice (36.19 mg/100 g) are higher than the white rice that contains 9.05 mg of magnesium in the same amount of 100 g. This may explain the role of brown rice consumption in regulating blood sugar control as magnesium is able to decrease insulin resistance by activating the autophosphorylation of insulin receptors (tyrosine kinase receptors) (Weg, 2022).

Based on the findings of Sun, et al. (2010), daily ingestion of 50 g of cooked brown rice may decrease the risk of diabetes mellitus by 16%. Improvement in postprandial blood glucose control following the daily intake of brown rice was also proven by Nakayama, et al. (2017). The authors reported a significant ($p < 0.05$) reduction in the post-meal sugar level in blood plasma by 21.5 mg/dL after the consumption of brown rice twice a day for 8 weeks. A significant ($p < 0.05$) decline in the indicator of blood glucose control, hemoglobin A1c (HbA1c) by 1.1% after the similar duration of brown rice intake was also reflected among 18 diabetic mellitus patients from St Marianna University Hospital with constant doses of daily insulin injection during the 8-week study. However, most of the research carried out have been focusing on short-term study. There is still lack of long-term trial study investigating the therapeutic effects of dietary fibers in brown rice with duration of up to years (Mao, et al., 2021).

Promoting Heart Health

Plant lignans such as matairesinol and lariciresinol are found in whole grains such as brown rice (14 µg/100 g). These compounds exhibit therapeutic functions on lowering the risk of cardiovascular diseases after being converted to mammalian lignans by gut microbiota (Peterson, et al. 2011). Mammalian lignans such as enterodiol (54%) and enterolactone (46%) were found to exert the hypocholesterolemia effect by preventing plaque formation in arteries which may subsequently lead to vasoconstriction or stenosis, the contributing factor to the disease of atherosclerosis (Liu, 2007; Pandey, Lijini and Jayadeep, 2017). The evidence on the positive correlation between the intake of brown rice (100

g/day) and the increased concentration of mammalian lignans in blood plasma by 37% was illustrated in a cross-sectional study done by Johnsen and his co-workers (2004) which involved 857 postmenopausal women. In addition to the blood cholesterol (LDL) lowering effect, decreasing vascular inflammation and aortic stiffness by mammalian lignans with high antioxidant activity were also verified in a research done on 781 middle-aged men and postmenopausal women by Pellegrini and his colleagues (2009). This further justified the potential of lignan in brown rice in reducing risk of cardiovascular disease.

2.1.6 Antioxidative Properties

Phenolics are the major phytochemicals found in brown rice which exhibit antioxidative properties and offer several health benefits for humans such as anticancer, anti-inflammatory and cardioprotective effects. Molecular structures of phenolics are in the form of benzene or aromatic ring(s) with the attachment of at least one hydroxyl group (Ravichanthiran, et al., 2018). Phenolic compounds are mainly constituted in the bran layer and germ of brown rice in the form of free, soluble, and bound phenolics (Ansari, 2020). Bio-accessibility of free phenolic compounds renders them the ability of being absorbed in small intestines. On the other hand, intact bound phenolics will pass through the small intestines until reaching the colon or large intestine for an interaction with the gut microbiota to balance the Firmicutes/Bacteroidetes (F/B) ratio which helps in controlling the intestinal inflammation (Martínez, et al., 2013). Table 2.1 classifies the types of phenolic compounds in brown rice with their respective concentrations and functions.

Table 2.1: Types, concentrations, and functions of phenolic compounds in brown rice.

Phenolic Compounds	Examples		Concentration	Functions	References
Phenolic Acids	Hydroxy-cinnamic acids (bound)	*Trans*-ferulic acid	161–375 µg/g	Inhibiting cell death, promoting heart health by inhibiting palate aggregation, and scavenging free radicals.	Rezaeiroshan, et al. (2022).
		Cis-ferulic acid	20.8–83 µg/g		
		Trans-p-coumaric acid	35.5–81.5 µg/g	Acting as anticancer agent by reducing the division of cancerous and tumor cells.	Kruszewski, et al. (2019).
	Hydroxy-benzoic acids	Vanillic acid	2.7–4.7 µg/g	Exhibiting anti-diabetic properties by decreasing blood glycemic level in diabetic groups.	Ingole, et al. (2011)
		Syringic acid	0.47–2.5 µg/g	• Preventing cerebral ischemia and neurological degeneration. • Acting as hepatoprotective agent by quenching liver-damaging free radicals.	Cheemanapallia, et al. (2018).

Table 2.1 (continued): Types, concentrations, and functions of phenolic compounds in brown rice.

Phenolic Compounds	Examples	Concentration	Functions	References
Flavonoids (free)	Quercetin	3.3–6.5 µg/g	• Exhibiting cardioprotective and anti-cancer effect. • Eliminating reactive oxygen and nitrogen species.	Ademosun, et al. (2016).
	Kaempferol	1.3–3 µg/g	• Displaying antioxidative properties by scavenging superoxide radicals. • Exhibiting anti-inflammatory properties by inactivating inflammation-inducing enzymes. For instance, cyclooxygenase.	Montaño, et al. (2011).
Proanthocyanin	Catechin	4.1–8.9 µg/g	• Quenching reactive oxygen species to prevent lipid oxidation in cells. • Retarding the activity of pro-oxidant enzyme to prevent oxidative stress and related diseases such as cardiovascular disease.	Bernatoniene and Kopustinskiene (2018).

2.1.7 Types of Brown Rice

Types of brown rice are further classified into four categories, namely long grain, medium grain, short grain, and light brown rice. They are different in the contexts of length, color, and intensity of nutty flavor. Due to their dissimilarity in the cooking time with the same ratio of rice to water (1:1.5–2), the texture of each type of cooked brown rice and types of dishes prepared may be varied from one another. Table 2.2 outlines the comparison between length, sensory attributes, cooking time and types of dishes that can be made from the four different types of brown rice.

Table 2.2: Comparison between long-grain, medium-grain, short-grain, and light brown rice (Kerkar, 2019; Longnecker, 2021).

Types / Properties	Long Grain	Medium Grain	Short Grain	Light Brown
Length of Kernels	6.61–7.50 mm	5.51–6.60 mm	< 5.50 mm	6.61–7.50 mm
Sensory Attributes	Firm, well-separated cooked brown rice grains with nutty flavor and chewy texture.	Texture of cooked rice is soft and moist.	Cooked brown rice has a creamy and sticky texture with sweet and malty flavor.	Cooked rice is light brown with light earthy flavor but has no chewy texture due to removal of 50% of bran.
Cooking Time	45 minutes	4-hour soaking with 15–20-minute cooking	Soaking overnight and cooking for 25 minutes	20 minutes
Types of Dishes	Pilaf, casseroles, and fried rice	Soup, paellas, and salads	Risotto and puddings	

2.2 Cooking Methods

2.2.1 Steaming

Steaming is a thermal preparation method which is applicable to a variety of foods such as rice, meat, fish, and vegetables. The science of steaming underlies the reliance of moist heat to be uniformly distributed throughout the foods until the foods are cooked (Andersson, et al., 2022). Hence, this necessitates a sufficient volume of water in the steaming pot that is brought to boiling by supplying heat to the water via convection. Once the boiling process is initiated, water molecules gain enough energy to overcome the strong hydrogen bonds holding them together. Therefore, water molecules escape into the vapor phase and vaporize into steam as a source of the required moist heat (Ilic, Petrovic and Stevanovic, 2019). The main difference between boiling and steaming foods can be portrayed in the setup of foods in the cooking pot. For boiling method, the foods will be directly submerged in boiling water during the cooking process. However, steaming foods may require a steamer tray where the bowl containing rice and water in an appropriate ratio, for instance, brown rice to water in the ratio of 1:2 will be placed on (Gavin, 2020). Then, water is filled into the steamer up to the level of the steamer tray, followed by boiling the water for steaming foods. The predetermined steaming duration for a particular food is only counted from the time when the water is boiled (Yao, 2009).

2.2.2 Frying

In contrast to steaming which uses moist heat, dry heat is required for frying due to the introduction of hot oil for the frying process (Dreeling, Allen and

Butler, 2000). The process of frying foods is divided into several stages. Xu, et al. (2020) point out that in the first stage, the heat is transferred from the flame to the oil and to foods by conduction, causing the rise in the surface temperature of foods to around 100°C. Hence, the elevated temperature results in the evaporation of water from the food interior in the following stage. To avoid complete dehydration of foods, a crust may be formed on the food surface for moisture retention (Oke, et al., 2017). In the subsequent deterioration stage, the occurred Maillard reaction, starch gelatinization and protein denaturation affect the color, flavor, and texture of foods when the heat is circulated within the foods by convection until the food is completely cooked (Schiffmann, 2017).

Frying methods can be classified into four common types, namely deep frying, pan frying, stir-frying and air frying that utilize the same principle but differ in the amounts of oil and utensils used. For deep frying, foods are fully immersed in hot oil (120°C–180°C) in a deep fryer. This marks a difference with pan frying or shallow frying and stir-frying where only little amount of oil that is sufficient to cover one-third of food pieces is required to fry the foods using a frying pan (Garayoa, et al., 2021). Therefore, foods are usually cut into thin slices in pan frying, while in stir-frying, the foods will be constantly stirred or tossed in the frying pan or wok for a quick and uniform heat transfer during the frying process (Berk, 2018). Air frying is conducted in an air fryer that is equipped with a heating element positioned close to the foods to supply heat by radiation. There is also a fan to circulate hot air (200°C) by convection in the heating chamber where the food is placed, to evenly transfer the heat all over the surface of food and cook the food (Arafat, 2014).

2.3 Comparison between Cooking Oils – Palm and Coconut Oils

2.3.1 Processing of Palm and Coconut Oils

Origin of palm fruit used for the extraction of palm oil is from the oil palm tree, *Elaeis guineensis* (Boateng, et al., 2016). In the initial stage of palm oil processing, threshing for the detachment of palm fruits from bunch stems is accomplished manually or mechanically using a rotating drum. In large-scale production, sterilization at 130–160°C with moist heat for 60–90 minutes is also performed to soften the fruit stem, thus stripping fruits from the bunches. Sterilization also serves for the purposes of inhibiting lipid hydrolysis as well as easing oil digestion and extraction (Tan, et al., 2021). Owolarafe, Faborode and Ajibola (2002) mentioned that autoclaving at the pressure of 40 psig for one and half hour resulted in the inactivation of lipase in palm fruit to suppress the formation of free fatty acids that may lead to fat rancidity. With the application of heat, disruption of pulp structure facilitates the separation of fibers in the later stage of digestion. Due to the softening of fruit mesocarp during sterilization, oil contents in the mesocarp can also be released easily. During sterilization, it is essential to introduce steam into the sterilizer for air removal. Otherwise, buildup of air may inhibit heat transfer and initiate the oxidation of fatty acids at high temperature with the exposure to oxygen (Biodun, et al., 2021).

Digestion of palm fruits is aimed for the destruction of exocarp of fruits to release palm oil from the palm fruits by pounding the fruits using rotating beater arms in a steam-heated digester (Tan, et al., 2021). The oil extraction is achieved in the process called pressing, using wet or dry methods which respectively rely

on the use of hot water or mechanical pressure (hydraulic press in batch system and screw press in continuous system) to expel oil from the digested mesh that consists of oil, water, press cake and nuts. Since the extracted palm oil may be contaminated with other impurities such as cell debris and fibers, clarification is required to remove the impurities. During the clarification process, addition of hot water is based on the ratio of 3 (water): 1 (output mixture) (Mba, Dumont and Ngadi, 2015). Beside diluting the thick mixture, water addition allows the separation of impurities according to the difference in the density with respect to the oil, by heating the oil mixture to 87°C. After a settling time of 2 hours in the clarification tank, oil with lower density will float as a layer on top of the water. After skimming off the oily layer and purifying the oil in centrifuge machine, the palm oil is dried in a vacuum dryer to decrease the moisture content to 0.15%–0.25% to minimize autocatalytic hydrolysis of the palm oil during storage, thus extending its shelf life without unpalatable rancid smell and taste (Juliano and Knoerzer, 2016).

Coconut oil is commonly derived from the coconut tree with the species of *Cocos nucifera* (Boateng, et al., 2016). Processing of coconut oil begins with de-husking to remove the husk from coconut fruits by cracking, to obtain the kernel. Drying of kernel lowers the moisture content from 50% to 6%–8%. In traditional processing, drying is performed under the hot sun for at least three days until the desired moisture content is reached. However, modern technology such as direct heat method can also be applied to increase the efficiency of drying (Savva and Kafatos, 2016). To avoid any small fluctuation in the moisture content of dried kernel (copra) due to the humidity in the environment,

reheating of copra is performed in a dryer. After piecing copra using an electric cutter which is followed by roasting with steam for at least 45 minutes at 70°C, the copra is sent to an oil expeller for oil extraction from the copra. The cake residue is also removed from the extracted oil by a vibrator (Pham, 2016).

Physical or chemical refining process of coconut oil is intended for impurities removal, to produce a refined coconut oil that suits for human consumption. Refining of crude coconut oil by physical mean involves the use of aqueous phosphoric acid (0.1% w/v) to remove phospholipids. Natural antioxidant such as gamma-tocopherol in 50 ppm is added to prevent fat oxidation during storage (Liu, et al., 2019). After pre-heating the coconut oil to 85°C for half an hour, mixture of activated carbon and bleaching earth (1:10) is used for oil bleaching process which is carried out at 93°C for 25 minutes. Lastly, deodorization of coconut oil is conducted by steam at 230°C under vacuum for an hour, producing a refined coconut oil without coconut odor (Siriphanich, et al., 2011).

2.3.2 Nutritional Compositions of Palm and Coconut Oils

Nutritional compositions of palm oil deviate from coconut oil in the main aspect of fatty acids profiles. Boateng, et al. (2016) stated that 100 g of palm oil consisted of 48.0 g and 46.0 g of saturated and unsaturated fatty acids, respectively. Coconut oil wise, 86.5 g of saturated fatty acids are contained in 100 g of coconut oil which are around 11.5 times higher than the contents of unsaturated fatty acids (7.5 g). Palmitic (44%) and lauric acids (49%) constitute the major contents of saturated fatty acids in palm and coconut oils, respectively

(Assuncao, et al., 2008; Mancini, et al., 2015). Table 2.3 shows the differentiation between the fatty acid contents of palm and coconut oils.

Table 2.3: Fatty acids profiles of palm and coconut oils (Assuncao, et al., 2008; Elsheikh, et al., 2013; Mancini, et al., 2015).

Fatty Acids	Palm Oil	Coconut Oil
Saturated Fatty Acids		
Caprylic Acid (C8)	Nil	8%
Capric Acid (C10)	Nil	7%
Lauric Acid (C12)	0.20%	49%
Myristic Acid (C14)	1.18%	16%
Palmitic Acid (C16)	44%	9%
Margaric Acid (C17)	0.10%	Nil
Stearic Acid (C18)	4.50%	3%
Arachidic Acid (C20)	0.10%	Nil
Monounsaturated Fatty Acids		
Palmitoleic Acid (C16:1)	0.07%	Nil
Oleic Acid (C18:1)	39.20%	6%
Gadoleic Acid (C20:1)	0.15%	Nil
Polyunsaturated Fatty Acids		
Linoleic Acid (C18:2)	10.10%	2%
Linolenic Acid (C18:3)	0.40%	Nil

2.3.3 Antioxidative Properties of Palm and Coconut Oils

Palm and coconut oils contain antioxidants such as carotenoids and vitamin E. Carotenoids are natural pigments found in plants that contribute to the red, yellow, and orange colors. Synthesis of carotenoids may also occur in the cyanobacteria and phototropic bacteria (Green and Parson, 2003). The basic structure of carotenoids is in the form of isoprene that comprises of up to 40 carbon atoms. Carotenoids can be grouped into two major groups based on the difference in the chemical structure, especially at the end of the basic structure of isoprene (Liu, 2007). Beta-carotene, gamma-carotene, and lycopene fall under the carotene group. They can be further subdivided into acyclized and cyclized carotenes in which beta-carotene with an ionone ring at each end is termed as cyclized carotene while acyclized structure describes the absence of ionone ring in the structure of lycopene. The second large group of carotenoids possesses hydroxyl functional groups at the ionone ring, being referred to as xanthophyll, lutein and zeaxanthin that are differentiated according to the number of hydroxyl groups attached (Cvetkovic and Nikolic, 2017).

Chiu, Coutinho and Gonçalves (2009) reported that carotenoids were contained in palm oil in the range of 500–700 mg/kg with mostly alpha and beta-carotene that made up at least 80% of total carotenoids, which explained the intense yellow color of palm oil. On the other hand, 0.34 mg/kg of carotenoids were found in coconut oil (Kumar and Krishna, 2015). The presence of carotenoids in palm and coconut oils is valuable due to their provitamin A and antioxidant activity. Antioxidant activity of carotenoids is exhibited against reactive oxygen

species such as singlet oxygen and hydroxyl radicals to reduce oxidative stress (Stahl and Sies, 2003). Biological functions of carotenoids as an antioxidant are attributed to their electron-rich polyene rings with conjugated double bonds that help in stabilizing free radicals via electrophilic attack. Carotenoids also act as the scavenger of peroxyl radicals by converting the carotenoid structure such as beta-carotene to carbonyl and dialkyl peroxides via the heterolysis process (Dewanjee, et al., 2021). The mechanism of carotenoids in quenching peroxyl radical also takes place through the hydrogen abstraction method by transferring an allylic hydrogen atom to the radical to neutralize the radical (Fiedor and Burda, 2014).

Vitamin E is designated as a fat-soluble or lipophilic antioxidant. Basic structure of vitamin E illustrates the presence of a phytol chain with a chromanol ring bonded at one end (Woollard and Indyk, 2003). The degree of saturation of phytol chain decides the groups of vitamin E, either tocopherol or tocotrienol, whereby tocopherol and tocotrienol consist of a saturated and unsaturated phytol chain, respectively. A difference in the chemical structure, particularly in the chromanol ring also leads to the subdivision of tocopherol and tocotrienol into four different stereoisomers (Kamyab, 2021). In this context, alpha isomer of tocopherol and tocotrienol is composed of a chromanol ring with the attachment of three methyl groups (-CH_3) while a methyl group is attached to the position R_3 of chromanol ring in the delta isomer. Beta and gamma isomers of tocopherol and tocotrienol illustrate a different position where the two methyl groups are bonded to the chromanol ring, in which the attachments of methyl

groups are observed in the position R_1 and R_3 for beta isomer, and R_2 and R_3 for gamma isomer (Liu, 2007).

The antioxidative properties of tocopherol and tocotrienol are signified by the presence of hydroxyl group in the chromanol ring. In the interaction with free radicals, the hydrogen atom of hydroxyl group is readily transferred to the free radicals to stabilize the free radicals via resonance stabilization (Liu, 2007). Ahsan and his co-workers (2014) deduced that tocopherol and tocotrienol were effective in scavenging both reactive oxygen species (ROS) and reactive nitrogen species (RNS). An *in-vivo* study carried out also showed the anti-inflammatory properties of vitamin E in inactivating the inflammation-inducing enzymes such as 5-lipoxygenase which catalyzed the synthesis of leukotriene which was linked to inflammatory diseases. Besides, inhibition of pro-inflammatory nuclear factor kappa B pathway to suppress the production of pro-inflammatory cytokines was also responsible for the anti-inflammatory and antioxidant activities of tocopherol and tocotrienol (Liu, 2017).

The total carotenoids and vitamin E contents in palm oil are ranging from 500–700 ppm and 800–1000 ppm, respectively, which are higher than the coconut oil with 0.34 ppm of total carotenoids and 4.9 ppm of vitamin E (Barnaby, et al., 2018; Kumar and Krishna, 2015). Table 2.4 shows the comparison between the total carotenoids and vitamin E contents in palm and coconut oils.

Table 2.4: Difference between the contents of carotenoid and vitamin E in palm and coconut oils (Barnaby, et al., 2018; Kumar and Krishna, 2015; Mancini, et al., 2015).

Type of Antioxidants	Concentration (mg/kg)	
	Palm Oil	**Coconut Oil**
Carotenoid	581	0.34
Vitamin E	843	4.9
α-Tocopherol	186	1.6
α-Tocotrienol	170	2.0
γ-Tocopherol	-	0.7
γ-Tocotrienol	387	0.6
δ-Tocotrienol	100	-

2.3.4 Potential Health Benefits of Palm and Coconut Oils

Improving Brain Health

Numerous studies conducted in recent years have shown the potential of vitamin E, specifically tocotrienol in palm and coconut oils in promoting brain health due to its antioxidant activity. This is done by protecting brain cells against oxidative stress to lower the risk of neurodegeneration in elderly. Neurodegenerative disorder such as Alzheimer's disease is initiated by amyloid-β (Aβ) that is accumulated in the cerebral cortex of brain, leading to the impairment of neuronal function (Hardy and Selkoe, 2002). Hence, Ibrahim, et al. (2017) had performed an *in-vivo* study on double transgenic mice to examine the effect of supplementation of vitamin E from palm oil on the progression of amyloid-β aggregation. The results reflected that 10-month

supplementation of alpha-tocotrienol (195 mg/g), alpha-tocopherol (166 mg/g), gamma-tocotrienol (256 mg/g), and delta-tocotrienol (76 mg/g) from palm oil attenuated the immunoreactive depositions of amyloid-β in the brain of treated mice. In novel object recognition test, treated mice showed a higher recognition index than wild mice, indicating an enhanced function of cognitive system in the treated mice. Apart from the *in-vivo* studies on animals, clinical investigation was carried out on 121 patients diagnosed with dementia or white matter lesion. The authors reported that the patients supplemented with 200 mg of tocotrienols from palm oil twice a day recorded an attenuated progression of white matter lesion after 2 years of study (Gopalan, et al., 2014).

In terms of coconut oil, medium chain fatty acid such as lauric acid is believed to counteract early symptoms of Alzheimer's disease (Hewlings, 2020). This is achieved by inducing ketosis to synthesize ketone body as an energy source for neuronal and non-neuronal functions to offset the disrupted brain glucose metabolism caused by neurogenerative disease (Cunnane, et al., 2016). In this case, the result of the 3-week pilot study that was performed on 22 patients with Alzheimer's disease deduced that the patients provided with Mediterranean diet with coconut oil demonstrated a significant ($p < 0.05$) increase in the semantic and episodic memory as compared to the 22 control subjects (Orti, et al., 2018).

Exhibiting Provitamin Activity

Carotenoid contents in palm and coconut oils are claimed to exhibit provitamin A activity. This is essential for the patients with vitamin A deficiency, thus

alleviating the vitamin A deficiency related disease such as vision problem (Tan, et al., 2021). Carotenoid such as beta-carotene share a common molecular structure with vitamin A. Two molecules of retinal are yielded via the cleavage of double bond at the center of a beta-carotene molecule with the aid of β-carotene dioxygenase in the inner lining of small intestine (Toti, et al., 2018). By reducing retinal to retinol through hydrogenation by retinaldehyde reductase, the presence of saturated and unsaturated fatty acids in palm and coconut oils further enhances the bioavailability of retinol or vitamin A in intestinal tracts by 80% due to the lipid-solubility of vitamin A (Rao, 2000).

Several studies about the improvement of vitamin A status by carotenoids in palm and coconut oils had been done. Disease such as cystic fibrosis is associated with the malabsorption of lipophilic vitamins, one of which is vitamin A, with reduced levels of beta-carotenes in blood plasma (Back, et al., 2004; Sommerburg, et al., 2015). According to the study conducted on 11 patients with cystic fibrosis, daily intake of palm oil in the amount of 2–3 tablespoons which was equivalent to 1.5 mg of beta-carotene led to a significant ($p < 0.001$) increase in the concentration of retinol in blood plasma after 56 days (Sommerburg, et al., 2015). This indicated the bio-efficacy of carotenoids in palm oil in producing and facilitating the absorption of vitamin A in human body. The results were further complemented by the meta-analysis performed by Dong and his co-workers (2017), which portrayed that daily intake of at most 8 g of palm and coconut oils resulted in a significant ($p < 0.00001$) rise in the concentration of vitamin A in blood serum in the tested human subjects as compared to the placebo group. Moreover, it was revealed that palm oil showed

a similar effectiveness as vitamin A supplements in relieving vitamin A deficiency. This was evidenced by the study showing an improvement of 2.5 and 3.2 folds in the levels of provitamin A beta-carotene and alpha-carotene, respectively, in the breastmilk of 98 lactating mothers with daily intake of 15 mL of palm oil added to the breakfast meal (three-quartered cups of black beans). On the other hand, the growth of carotene concentrations by 1.6 folds was observed in the breastmilk of the lactating mothers with daily doses of 15 mg of beta-carotene capsules with same duration of 10 days (Canfield, et al., 2001).

2.4 Colorimetry

Initial impression of foods is influenced by visual attribute such as color. For color to be detected, a colored object, visible light (380 nm–770 nm), and an observer or the detector of colorimeter must interact with one another. Principle of colorimetry or color quantification is based on the human perception of color which combines the response of human's eyes (retina) and brain (Smith, 2014).

Instrumentation of colorimeter consists of a light source (illuminant), a monochromator with a tristimulus absorption color filter (red, green, and blue chroma) and slit, sample holder (granular material attachment for solid foods; Petri dish or cuvette for liquid foods) and a detector (Giri, 2022). For colorimeter which measures visible light, tungsten lamp is commonly used as the light source (Batra, 2018). Monochromator such as prism allows monochromatic light with a specific wavelength to pass into the samples being analyzed with the aid of a slit which eliminates the unwanted light from reaching

the samples (Giri, 2022). Once the light contacts with the samples, some incident lights are absorbed and reflected by the samples. Hence, the transmitted incident lights are detected by the detector such as photocells (Ly, et al., 2020). Transmission data which is expressed in the ratio of transmitted light intensity to incident light intensity is converted into tristimulus values by the microprocessor (Gupte, 2010). Total color difference (ΔE*) calculated using the formula $\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$ provides a measure of sample lightness, redness, and yellowness as compared to the reference (Lamberts, et al., 2006).

2.5 Texture Profile Analysis (TPA)

Food quality may be judged by sensory property such as texture. Throughout food processing, final food texture may be governed by process variables such as humidity, cooking temperature and duration (Fellows, 2017). Therefore, texture analysis must be in place as part of the quality control in food industries, which also acts as a complement to the sensory test conducted by sensory panels.

Textural parameters measured include hardness, cohesiveness, adhesiveness, viscosity, elasticity, fracturability, chewiness, and gumminess. Selection of texture profile analysis test is critically dependent on the types of foods to be tested (Peleg, 2019). Table 2.5 summarizes the types of texture profile analysis tests with their purposes, measured properties and common types of probes used.

Table 2.5: Types of texture profile analysis tests.

TPA Tests	Purposes	Measured Properties	Types of Probes	References
Puncture	To measure degree of ripening (fresh produces), spreadability (shortenings), and hardness (cheese and confectionery).	Stiffness, gel strength, consistency of semi solids, actuation force	2–10-mm diameter cylinder probe; ball probe; 1-mm diameter needle probe (crosshead speed of 10 mm/min to 30 mm/s)	Liu, Chao and Liu (2019).
Compression	To examine compressibility of viscoelastic foods such as confectionery and baked goods.	Firmness, Young's modulus, springiness, stress relaxation	10–80-mm diameter cylinder probe or 150-mm diameter compression platen with total surface area larger than samples (crosshead speed: 4 mm/s)	Paula and Silva (2014).

Table 2.5 (continued): Types of texture profile analysis tests.

TPA Tests	Purposes	Measured Properties	Types of Probes	References
Bending	To evaluate fracturability of bar/sheet shaped foods which underlies the moisture content of foods, turgor pressure of fresh produces and indication of how much shortenings are used in baked products.	Bend and flexure modulus, flexibility, brittleness, fracture force	3-point bend rig (crosshead speed of 1–120 mm/min); crisp fracture support rig; spaghetti flexure rig	Chen and Opara (2013).
Tensile and extensibility	To determine tensile properties of foods such as spaghetti and dough. To ensure endurability of food packaging materials in response to physical abuse during transportation and storage of foods.	Tensile strength, extensibility, tensile modulus, yield stress, yield strain	Tensile grips; self-tightening grips; extensibility rig	Kraithong and Rawdkuen (2021).

Apart from the types of probes that are selected specifically according to the analyzed samples, other instrumental settings such as test speed, strain, and trigger force are also taken into account while operating the texture analyzer. Pretest speed is defined as the descending speed of the probe (Srilakshmi, 2020). Initiation of the record of data for the generation of texture analysis graph occurs upon reaching the automatic trigger force (Peleg, 2019). Degree of compression of food samples by the probe will be based on the test speed and the travelling distance of the probe into the samples or strain expressed in the unit of percentage (Herrero, et al., 2008). Once the first compression is completed, the probe is then lifted up to the initial position at the same test speed. To be synonymous with humans' chewing action, the food samples will be compressed twice with exactly the same steps but with predetermined waiting time between the first and second compression for sample recovery, thus giving texture profile analysis the name as two-bite test (Rosenthal, 2010). Then, the ascending of the probe to the initial position at post-test speed signifies the accomplishment of the two-times compression (Peleg, 2019).

2.6 Antioxidant Assays

2.6.1 Total Phenolic Content (TPC) Assay

Total phenolic content assay is practised to evaluate the contents of phenolic compounds in samples, providing a measure of the antioxidant activity. TPC assay relies on the principle of redox reaction occurred between the reagent added, Folin-Ciocalteu, and phenolics present in the sample extracts being examined. In the alkaline condition created attributing to the presence of basic

solution of sodium carbonate, loss of a proton (H^+) from the phenolic compound leads to the formation of a phenolic ion (Martono, et al., 2019). Folin-Ciocalteu reagent composing of phosphotungstic ($H_3PW_{12}O_{40}$) and heteropoly phosphomolybdic acids ($H_3PMo_{12}O_{40}$) act as oxidizing agents by accepting electrons from the hydroxyl groups of phenolic ions of polyphenols for phenol oxidation (Almey, et al., 2010). Meanwhile, a reduction of the phosphotungstic and phosphomolybdic acids in Folin-Ciocalteu takes place, in which the molybdenum with a charge of +6 (yellow) is reduced to the charge of +5 (blue) due to the receive of an electron from the phenolate anions. As a result, a stable molybdenum-tungsten complex (blue) is produced (Echegaray, et al., 2021). Therefore, in the presence of high concentration of phenolic ions in the sample extracts, more heteropoly acids in Folin-Ciocalteu are converted to the stable blue complex following the redox reaction. Hence, solution with high intensity of blue color is produced. Total phenolic contents in the samples are determined by measuring the absorbance of the colored complex formed using a UV-Vis spectrophotometer at the wavelength of 765 nm against a phenolic compound such as gallic acid as the reference standard (Martono, et al., 2019).

2.6.2 Total Flavonoid Content (TFC) Assay

Total flavonoid content assay is aimed to determine the amounts of flavonoids, such as anthocyanins, flavones and flavonols in the tested samples, using aluminum chloride (Ahmed and Iqbal, 2018). The reaction between aluminum chloride and flavonoids gives rise to the production of a stable colored complex whose absorbance is measurable with UV-Vis spectrophotometer in a specific

range of wavelength, with the maximum of 510 nm. For instance, an orange complex that is stable in acidic condition is formed when aluminum ion of aluminum chloride reacts with the keto group and hydroxyl group at carbon number 4 and carbon number 3 or 5, respectively, to generate structure of O–Al^{+}–O in the molecular structure of flavonols and flavones (Sepahpour, et al., 2018). Besides, with the addition of sodium nitrite (nitrating agent) prior to introducing aluminum chloride into the solutions of sample extracts, a reaction may be targeted on the 1,2-dihydroxyl groups (diols) in the aromatic rings (A or B) of flavonoids, thus forming an acid-sensitive complex in an alkaline environment due to the presence of sodium hydroxide solution added (Shraim, et al., 2021). Since quercetin is categorized in the class of flavonoids, quercetin is frequently used as reference standard for the estimation of total flavonoid contents in the sample extracts (Tristantini and Amalia, 2019).

2.6.3 DPPH Free Radical Scavenging Activity Assay

DPPH or 2,2-diphenyl-1-picryl-hydrazyl is a stable free radical with an odd electron on nitrogen radical and delocalized electrons along the chemical bonds (Liang and Kitts, 2014). Antioxidants can scavenge free radicals such as DPPH radical by donating a free hydrogen atom to the radical, forming a stable, diamagnetic non-radical, 2,2-diphenyl-1-picryl-hydrazine via hydrogen atom transfer (HAT) mechanism. Owing to their proton donating abilities, DPPH radical scavenging activity gives a denotation of the antioxidant activity. Reduction of DPPH radicals to DPPH hydrazine leads to the color change of solution from purple to pale yellow, decreasing the absorbance at the

wavelength of 517 nm (Gangwar, et al., 2014). Hence, an inversely proportional model exists between the absorbance of solution at the specified wavelength and radical scavenging activity of antioxidants or antioxidant activity. The expression of antioxidant activity based on DPPH assay is in the form of IC_{50} which indicates the concentration of antioxidants required to scavenge DPPH free radical with the percentage of up to 50% (Jadid, et al., 2017). In this context, a low IC_{50} indicates a high antioxidant activity.

2.6.4 Reducing Power Assay

Antioxidants are reducing agents that readily donate electrons to other molecules for the reduction of the particular compound. According to Jayanthi and Lalitha (2011), in reducing power assay, an electron will be transferred to potassium ferricyanide to reduce Fe^{3+} to Fe^{2+}, generating potassium ferrocyanide. Further reaction between potassium ferrocyanide and ferric chloride then produces a Fe^{2+}-Fe^{3+} colored complex. The occurrence of the reaction results in the color change of solution from colorless to blue. In contrast to the concept of DPPH assay, an increasing absorbance at the wavelength of 700 nm illustrates the high reducing power or high antioxidant activity which corresponds to the role of antioxidants as an electron donor (Bhalodia, et al., 2013). In reducing power assay, the term EC_{50} or the concentration of antioxidants needed to reach 50% of the antioxidative effect is commonly used as an indicator of the antioxidant activity whereas a negative correlation is observed between the two variables (Chen, Bertin and Froldi, 2013).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

EcoBrown's unpolished brown rice, Alif branded palm oil, and coconut oil from the brand, Medella, Malaysia, were purchased from the local supermarket, Lotus's Kampar.

3.2 Equipment

Table 3.1: Lists of equipment used with their manufacturers.

Equipment	Manufacturers
Centrifuge machine	Dynamica, Malaysia
Colorimeter	Konica Minolta, Japan
Drying oven	Memmert, United States
Electronic balance	Mettler Toledo, United States
Hot plate	Thermo Scientific, United States
Laboratory grinder	IKA, Malaysia
Micropipettes	Gilson, United States
Microplate reader	BMG Labtech, Germany
Orbital shaker	Infors HT, Switzerland
pH meter	Mettler Toledo, United States
Texture analyzer	Stable Micro Systems, United Kingdom
Vortex mixer	Scientific Industries, United States

3.3 Chemicals

Table 3.2: Lists of chemicals used with their manufacturers.

Chemicals	Manufacturers
Aluminum chloride anhydrous	Friendemann Schmidt, United States
Ascorbic acid	HmbG, Malaysia
DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) powder	Sigma-Aldrich, United States
Ferric chloride hexahydrate	Merck, Germany
Folin-Ciocalteu's phenol reagent	Chemiz, Malaysia
Gallic Acid	Alfa Aesar, United States
Methanol	Emsure, Germany
Potassium ferricyanide	HiMedia, India
Quercetin	Sigma-Aldrich, United States
Sodium carbonate	Merck, Germany
Sodium hydroxide pellet	Merck, Germany
Sodium nitrite	Bendosen, Malaysia
Sodium phosphate dibasic dodecahydrate	Bendosen, Malaysia
Sodium phosphate monobasic dihydrate	Bio Basic, Canada
Trichloroacetic acid	Bendosen, Malaysia

3.4 Overview of Methodology

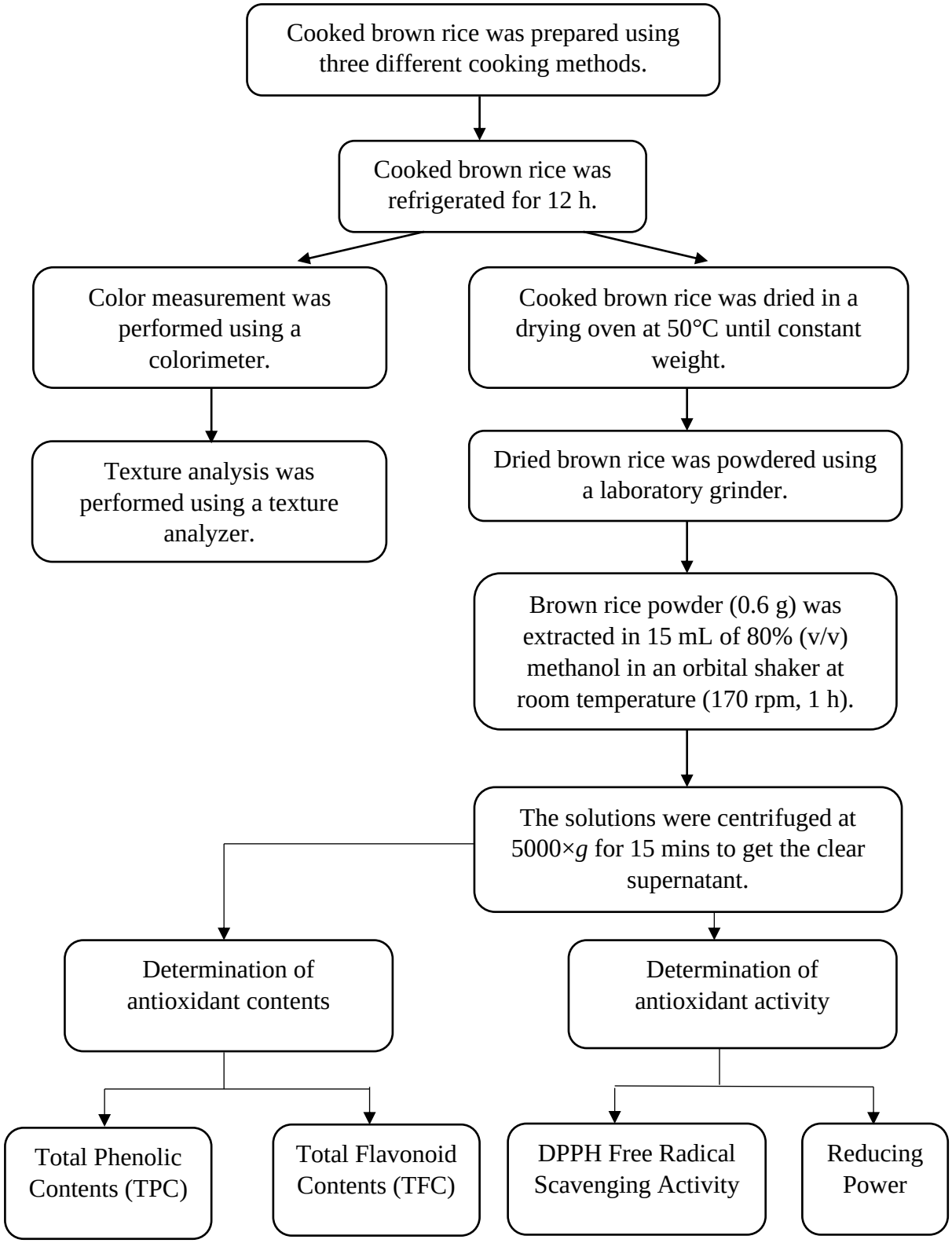

Figure 3.1: Overview of methodology of the study.

3.5 Preparation of Cooked Brown Rice Samples

Brown rice was cooked using three different cooking methods and two different types of cooking oils (palm and coconut oils). The design of cooking methods was adopted from the methods described by Kaur, et al. (2015) with slight modifications. First, raw brown rice (30 g) was rinsed with tap water and dried using paper towels. In cooking method I, rice mixed with 0.9 g of palm oil was stir-fried in a preheated frying pan at medium heat (level 5) for 1 min. The fried rice was then steamed in 50 mL of filtered water in a bowl in the steaming pot for 40 mins at medium heat (level 6). In cooking method II, after adding 0.9 g of palm oil to the raw brown rice, the brown rice was directly subjected to steaming with the same steaming conditions as in the cooking method I. In cooking method III, after steaming 30 g of raw brown rice in 50 mL of filtered water for 40 mins at medium heat (level 6), the steamed rice was immediately mixed with 0.9 g of palm oil and stir-fried in a preheated frying pan at medium heat (level 5) for 1 min. The steps in each cooking method were repeated for control (non-oil treated) and coconut oil. The samples of cooked brown rice were refrigerated for 12 h.

3.6 Color Measurement

Color measurement of cooked brown rice was performed following the protocol described by Lamberts, et al. (2006) with minor modifications. First, the colorimeter was calibrated. The cooked brown rice (1 g) was placed into a clean Petri dish. The color of cooked brown rice was measured by the colorimeter using CIE Lab scales. The measurement was performed in duplicates in two batches. The total color difference between control and oil-treated brown rice was calculated using the Equation 3.1:

Equation 3.1: $\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$

3.7 Texture Analysis

The refrigerated rice was put at room temperature for 2 h prior to the texture analysis. The methods of texture analysis described by Tao, et al. (2012) were applied with slight modifications. A 35-mm diameter cylinder probe was used. The height and force of texture analyzer were first calibrated. After calibration, the cooked brown rice with the weight of 1 g was evenly placed on the center of base plate. The hardness and stickiness of cooked brown rice were analyzed using the TA-XT texture analyzer based on the specific settings. For instance, the pre-test speed and test speed were set at 0.5 mm/s while 2 mm/s was specified for the post-test speed. Auto trigger force of 0.05 N with strain of 90% and time of 5 s was set. The texture analysis of cooked brown rice was conducted in duplicates in two batches.

3.8 Phytochemicals Extraction

The cooked brown rice was dried in the drying oven at 50°C until constant weight (Bait, et al., 2021). A laboratory grinder was used to powder the dried brown rice. The powder of dried rice samples was extracted using the methods developed by Chakuton, Puangpronpitag and Nakornriab (2012) and Thuengtung and Ogawa (2019) with slight modifications. A total of 0.6 g of powder was extracted in 15 mL of 80% (v/v) methanol in an orbital shaker at room temperature for an hour. The speed of the shaker was set at 170 rpm. The solutions were centrifuged in a centrifuge machine at 5000×g for 15 mins. The centrifuged extract solutions were kept at 4°C until analysis.

3.9 Total Phenolic Content (TPC) Assay

The extracts of cooked brown rice samples were assessed for the total phenolic content using the methods published by Priyanthi and Sivakanesan (2021) with minor modifications.

3.9.1 Preparation of Gallic Acid Standard Solutions

Gallic acid powder (5 mg) was dissolved in distilled water (10 mL) to prepare a gallic acid stock solution (500 mg/L). With distilled water as the diluent, serial dilution was performed as shown in Figure 3.2 to prepare gallic acid standard solutions with concentrations of 100 mg/L, 80 mg/L, 60 mg/L, 40 mg/L, and 20 mg/L. The solutions were prepared in duplicates and covered with aluminum foil.

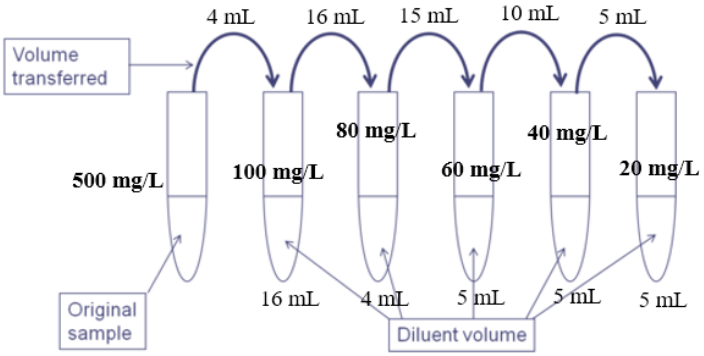

Figure 3.2: Serial dilution of gallic acid standard solutions.

3.9.2 Folin-Ciocalteu's Test

One milliliter of distilled water was added to 2 mL of supernatant of the centrifuged extract. A total of 50 μL of the solution was pipetted using a micropipette and mixed with 0.5 mL Folin-Ciocalteu's (FC) phenol reagent. The mixture was mixed using a vortex mixer. After 3 mins, the solution was mixed with 400 μL of 75 g/L sodium carbonate (Na_2CO_3) solution and vortexed. The solutions were incubated at room temperature for 30 mins. Two hundred microliters of each solution were pipetted into each of the wells of an Elisa 96-well plate. Measurement of absorbance was performed at 765 nm against a blank. The steps were repeated for gallic acid standard solutions to generate a standard calibration curve for the calculation of total phenolic contents of the sample extracts (mg GAE/100 g DW). The analysis of the sample extracts was performed in duplicates in two batches.

3.10 Total Flavonoid Content (TFC) Assay

Total flavonoid content assay of the extracts of cooked brown rice samples was performed following the methods described by Priyanthi and Sivakanesan (2021) with slight modifications.

3.10.1 Preparation of Quercetin Standard Solutions

Five milligrams of quercetin powder were dissolved in distilled water (10 mL) to prepare a quercetin stock solution with concentration of 500 mg/L. Preparation of quercetin standard solutions (100 mg/L, 80 mg/L, 60 mg/L, 40 mg/L, and 20 mg/L) was carried out by serial dilution as previously described in Section 3.9.1. The solutions were prepared in duplicates and covered with aluminum foil.

3.10.2 Aluminum Chloride Colorimetric Assay

One milliliter of distilled water was added to 2 mL of supernatant of the centrifuged extract. Two hundred and fifty microliters of the solution were then pipetted using a micropipette and mixed with 1.25 mL of distilled water. Next, 75 μL of 5% (w/v) sodium nitrite ($NaNO_2$) solution was added to the mixture. The mixture was mixed with 150 μL of 10% (w/v) aluminum chloride ($AlCl_3$) solution after 5 mins. After 6-mins incubation at room temperature, the mixture was mixed with 500 μL of 1 mol/L sodium hydroxide (NaOH) solution. A total of 200 μL of each solution was pipetted into each well of the Elisa 96-well plate. Measurement of absorbance was performed at 510 nm against a blank. The steps

were repeated for quercetin standard solutions to generate a standard calibration curve to calculate the total flavonoid contents of the sample extracts (mg QUE/100 g DW). The analysis of the sample extracts was performed in duplicates in two separate runs.

3.11 DPPH Free Radical Scavenging Activity Assay

Methods of DPPH assay described by Priyanthi and Sivakanesan (2021) were used in this research with minor modifications.

3.11.1 Preparation of Solutions of Sample Extracts

Different concentrations of sample extract solutions were prepared from the supernatants of extracts (0.04 g/mL) according to Table 3.3, using distilled water as the diluent.

Table 3.3: Preparation of sample extract solutions with different concentrations.

Extract (mL)	0.125	0.25	0.375	0.5	0.625
Distilled water (mL)	0.875	0.75	0.625	0.5	0.375
Final concentration (mg/mL)	5	10	15	20	25

3.11.2 Preparation of Pure Ascorbic Acid Solutions

A 100 mg/mL pure ascorbic acid stock solution was prepared by dissolving 10 g of ascorbic acid powder in 100 mL of methanol. A series of pure ascorbic acid solutions (25 mg/mL, 20 mg/mL, 15 mg/mL, 10 mg/mL, and 5 mg/mL) was prepared from the stock solution by serial dilution with methanol as the diluent, as shown in Figure 3.3. The solutions were prepared in duplicates and covered with aluminum foil.

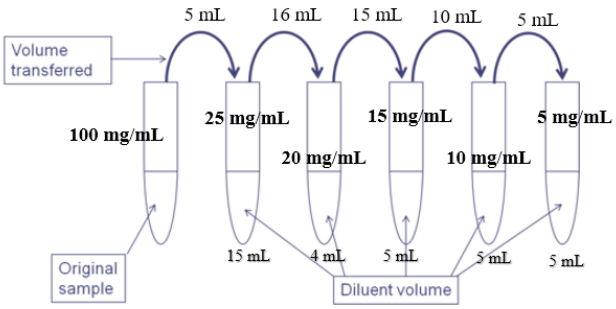

Figure 3.3: Serial dilution of pure ascorbic acid solutions.

3.11.3 DPPH Free Radical Scavenging Activity Assay

Methanolic DPPH solution (0.5 mM) was freshly prepared by dissolving 9.85 mg of DPPH powder in 50 mL of methanol. A volume of 1 mL of extract of each concentration was mixed with 250 μL of the freshly prepared methanolic DPPH solution (0.5 mM) using a vortex mixer. The step was repeated for negative control with 1 mL of methanol to replace the sample extract. After incubation of 30 mins in dark at room temperature, micropipette was used to aliquot 200 μL of each solution into each well of the Elisa 96-well plate. Measurement of absorbance was performed at 517 nm against a blank

(methanol). The steps were then repeated for pure ascorbic acid solutions. The analysis of the sample extracts was performed in duplicates in two batches.

Equation 3.2 was used to calculate the DPPH free radical scavenging activity (%) of sample extracts and pure ascorbic acid solutions. After plotting the graph of percentage of DPPH free radical scavenging activity against concentration, IC_{50} values (mg/mL) were identified from the graph by linear regression analysis.

Equation 3.2:

$$\text{DPPH free radical scavenging activity (\%)} = \frac{(A_{-\text{ve control}} - A_{\text{sample}})}{A_{-\text{ve control}}} \times 100\%$$

3.12 Reducing Power Activity Assay

The reducing power activity of sample extracts of cooked brown rice was determined using the protocols established by Priyanthi and Sivakanesan (2021) with slight modifications.

3.12.1 Preparation of Solutions of Sample Extracts

Preparation of different concentrations of sample extract solutions from the supernatants of extracts (0.04 g/mL) was performed using the steps as previously described in Section 3.11.1.

3.12.2 Preparation of Pure Ascorbic Acid Solutions

The stock solution of pure ascorbic acid (100 mg/mL) was prepared and diluted to the concentrations 25 mg/mL, 20 mg/mL, 15 mg/mL, 10 mg/mL, and 5 mg/mL using the steps as previously stated in Section 3.11.2. The solutions were prepared in duplicates and covered with aluminum foil.

3.12.3 Reducing Power Assay

First, 1 mL of 0.3 M phosphate buffer (pH 6.6) was added to 400 μL of sample extract of each concentration. One milliliter of 1% (w/v) potassium ferricyanide (K_3 [Fe (CN_6)]) solution was then added to the mixture. The steps were repeated for negative control with 400 μL of methanol to replace the sample extracts. After 20-mins incubation at 50°C, the mixture was mixed with 1 mL of 10% (w/v) trichloroacetic acid ($C_2HCl_3O_2$). Next, 2 mL of distilled water was added to 2 mL of the mixture. After adding 0.4 mL of 1% (w/v) ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) to the mixture, the mixture was incubated for 30 mins at room temperature. A volume of 200 μL of each solution was aliquoted into each of the wells of the Elisa plate. Measurement of absorbance was performed at 700 nm against a blank (methanol). The steps were repeated for pure ascorbic acid solutions. The analysis of the sample extracts was performed in duplicates in two batches.

Percentage of reducing power activity of sample extracts and pure ascorbic acid solutions was calculated using Equation 3.3. EC_{50} values (mg/mL) were

determined from the graph of percentage of reducing power activity against concentration by linear regression analysis.

Equation 3.3: Reducing power activity (%) = $\frac{(A_{sample} - A_{-ve\ control})}{A_{-ve\ control}} \times 100\%$

3.13 Statistical Analysis

Measurements were conducted in duplicates in two separate runs (n = 4). Results of measurements were indicated in the form of mean ± standard deviation. The differences between means for the factors of cooking methods and oils were analyzed separately by one-way analysis of variance (ANOVA) and Tukey's HSD multiple comparison test. However, the differences between the means of total color differences of palm oil and coconut oil treated brown rice were determined using an independent sample T-test. In DPPH and reducing power assays, means of IC_{50} and EC_{50} values between sample extracts and pure ascorbic acid were also compared using the independent sample T-test. At the confidence level of 95%, significant difference between means existed if $p < 0.05$, while $p > 0.05$ indicated insignificant difference between the means. Lastly, relationship between TPC, TFC, IC_{50} and EC_{50} values was analyzed using Pearson correlation at the significance level of 0.05. All statistical analysis were performed using SPSS software version 27.

CHAPTER 4

RESULTS

4.1 Color

L* (lightness), a* (redness-greenness), b* (blueness-yellowness) and ΔE* (total color difference) of control (non-oil treated), palm oil and coconut oil treated brown rice samples cooked by three different cooking methods were summarized in Table 4.1. In cooking method I, L* values of palm oil and coconut oil treated brown rice samples were significantly higher than that of the control. On the other hand, cooking method II significantly increased the L* value of brown rice cooked with palm oil. In cooking method III, L* values of control and oil-treated samples were insignificantly different with each other. For the control, palm oil and coconut oil treated brown rice, there was no significant difference ($p > 0.05$) between the L* values in all the three cooking methods. In terms of a* and b* values, cooking methods I, II and III did not have a significant effect ($p > 0.05$) on the readings of a* and b* of non-oil and oil-treated brown rice. Lastly, in all the three cooking methods, total color difference of brown rice cooked with palm oil was insignificantly different from that of the coconut oil treated rice samples. The total color differences of palm oil and coconut oil treated brown rice were not significantly affected by the three cooking methods.

Table 4.1: Lightness (L*), redness-greenness (a*), blueness-yellowness (b*) and total color difference (ΔE*) of control, palm oil and coconut oil treated brown rice samples cooked by three different cooking methods.

Measurement	Cooking Methods	Control	Palm Oil	Coconut Oil
L*	**I**	63.02 ± 1.05^{ay}	68.30 ± 0.54^{ax}	66.84 ± 2.24^{ax}
	II	65.20 ± 1.46^{ay}	69.61 ± 0.13^{ax}	66.47 ± 1.29^{ay}
	III	65.29 ± 2.02^{ax}	66.95 ± 2.28^{ax}	66.20 ± 3.07^{ax}
a*	**I**	0.23 ± 0.28^{ax}	0.42 ± 0.20^{ax}	0.27 ± 0.24^{ax}
	II	0.68 ± 0.29^{ax}	0.31 ± 0.22^{ax}	0.29 ± 0.10^{ax}
	III	0.37 ± 0.09^{ax}	0.52 ± 0.16^{ax}	0.76 ± 0.39^{ax}
b*	**I**	12.94 ± 0.62^{ax}	12.23 ± 1.63^{ax}	11.93 ± 2.00^{ax}
	II	14.92 ± 2.33^{ax}	14.58 ± 2.47^{ax}	13.96 ± 4.91^{ax}
	III	14.40 ± 0.79^{ax}	13.13 ± 3.19^{ax}	14.77 ± 2.76^{ax}
ΔE*	**I**	-	5.53 ± 0.35^{ax}	4.64 ± 1.11^{ax}
	II	-	4.93 ± 0.25^{ax}	4.33 ± 2.17^{ax}
	III	-	3.79 ± 1.47^{ax}	3.41 ± 1.80^{ax}

Presentation of data was in the form of mean ± standard deviation (n = 4).
[a]: Insignificant difference among data within column with same superscripts based on Tukey's HSD test ($p > 0.05$).
[x–y]: Significant difference among data within row with different superscripts based on Tukey's HSD test ($p < 0.05$).

4.2 Texture

Hardness and stickiness values of control (non-oil treated), palm oil and coconut oil treated brown rice samples cooked by three different cooking methods were documented in Table 4.2. In cooking methods I and II, the highest hardness values were shown in palm oil and coconut oil treated brown rice, while the control sample had the lowest hardness value. Cooking method III did not significantly affect the hardness of non-oil treated and oil-treated brown rice. For the control samples, no significant difference ($p > 0.05$) was noted between the hardness values in cooking methods I, II and III. For the brown rice cooked with palm oil and coconut oil, cooking methods I and II significantly increased the hardness of brown rice.

From the view of stickiness of cooked brown rice, in cooking methods I and II, control recorded significantly higher stickiness values than the oil-treated rice samples. In cooking method III, the stickiness of cooked brown rice was insignificantly different with each other. For the non-oil treated brown rice (control), no significant difference ($p > 0.05$) was indicated in the stickiness values between the three cooking methods. Lastly, the stickiness values of palm oil and coconut oil treated brown rice were comparable in cooking methods I and II.

Table 4.2: Hardness and stickiness of control, palm oil and coconut oil treated brown rice samples cooked by three different cooking methods.

Texture	Cooking Methods	Control	Palm Oil	Coconut Oil
Hardness (g)	**I**	13947.98 ± 145.23^{ay}	18833.95 ± 157.96^{ax}	18515.83 ± 186.66^{ax}
	II	12260.30 ± 159.26^{ay}	18953.64 ± 111.94^{ax}	17982.53 ± 130.81^{ax}
	III	13302.27 ± 98.70^{ax}	13869.37 ± 118.26^{bx}	13561.38 ± 116.56^{bx}
Stickiness (g)	**I**	3.48 ± 0.21^{ax}	2.45 ± 0.32^{by}	2.32 ± 0.12^{by}
	II	3.53 ± 0.27^{ax}	2.43 ± 0.40^{by}	2.61 ± 0.33^{by}
	III	3.32 ± 0.10^{ax}	3.28 ± 0.28^{ax}	3.11 ± 0.21^{ax}

Presentation of data was in the form of mean ± standard deviation (n = 4).
$^{a–b}$: Significant difference among data within column with different superscripts based on Tukey's HSD test ($p < 0.05$).
$^{x–y}$: Significant difference among data within row with different superscripts based on Tukey's HSD test ($p < 0.05$).

4.3 Total Phenolic Content (TPC)

Total phenolic contents of control, palm oil and coconut oil treated brown rice samples cooked by three different cooking methods were calculated from the gallic acid standard calibration curve (Figure 4.1). The total phenolic contents of cooked brown rice samples were shown in Table 4.3. In cooking method I, there was a significant ($p < 0.05$) increase in the total phenolic content of palm oil treated brown rice. In cooking method II, palm oil treated brown rice sample recorded higher total phenolic content, while the control had lower total

phenolic content. In cooking method III, there was no significant difference ($p > 0.05$) between the total phenolic contents of non-oil and oil-treated samples. For control samples, cooking method II significantly decreased the total phenolic content of brown rice. In terms of palm oil treated brown rice, the total phenolic content in cooking method I was significantly higher than the other two cooking methods. Lastly, for the brown rice cooked with coconut oil, the total phenolic contents in all three cooking methods were insignificantly different with each other.

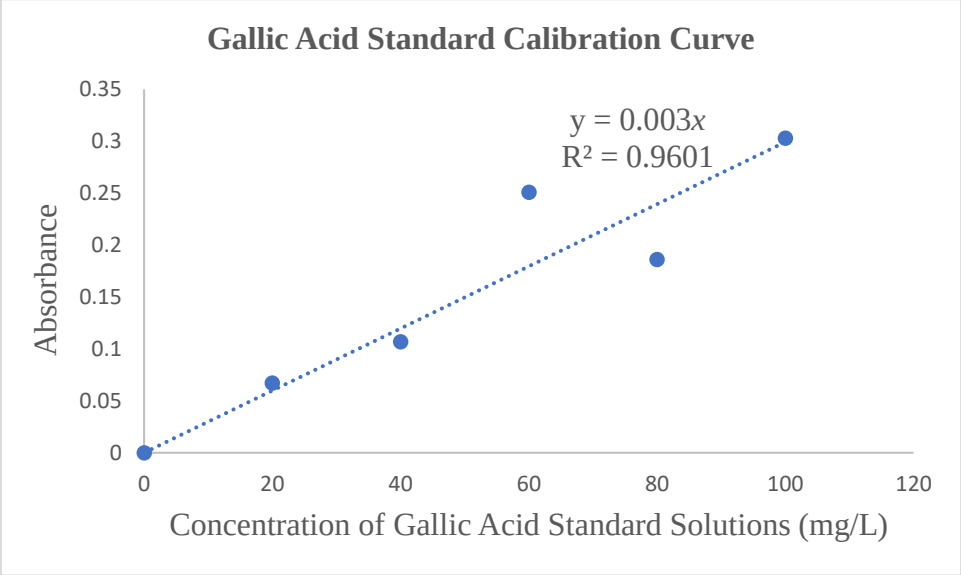

Figure 4.1: Relationship between absorbance values and concentrations of gallic acid standard solutions.

Table 4.3: Total phenolic contents of control, palm oil and coconut oil treated brown rice samples cooked by three different cooking methods.

Cooking Methods	Total Phenolic Content (mg GAE/100 g DW)		
	Control	**Palm Oil**	**Coconut Oil**
I	37.50 ± 8.90^{ay}	68.44 ± 6.24^{ax}	49.38 ± 9.49^{ay}
II	23.13 ± 2.98^{bz}	52.19 ± 4.38^{bx}	41.56 ± 6.72^{ay}
III	38.75 ± 5.68^{ax}	47.50 ± 3.68^{bx}	39.38 ± 4.84^{ax}

Presentation of data was in the form of mean ± standard deviation (n = 4).
[a–b]: Significant difference among data within column with different superscripts based on Tukey's HSD test ($p < 0.05$).
[x–z]: Significant difference among data within row with different superscripts based on Tukey's HSD test ($p < 0.05$).

4.4 Total Flavonoid Content (TFC)

Total flavonoid contents of control, palm oil and coconut oil treated brown rice samples cooked by three different cooking methods were calculated using quercetin standard calibration curve (Figure 4.2). The total flavonoid contents of cooked brown rice were noted in Table 4.4. In cooking method I, the total flavonoid contents of brown rice cooked with palm oil and coconut oil were comparable with each other. On the other hand, in cooking methods II and III, higher total flavonoid contents were observed in the palm oil treated brown rice samples, while the control samples showed lower total flavonoid contents. In terms of the control and brown rice cooked with palm oil, higher total flavonoid content was shown in cooking method III, while cooking method I with lower total flavonoid content was portrayed. However, there was no significant difference ($p > 0.05$) in the total flavonoid contents between the three cooking methods for the coconut oil treated brown rice.

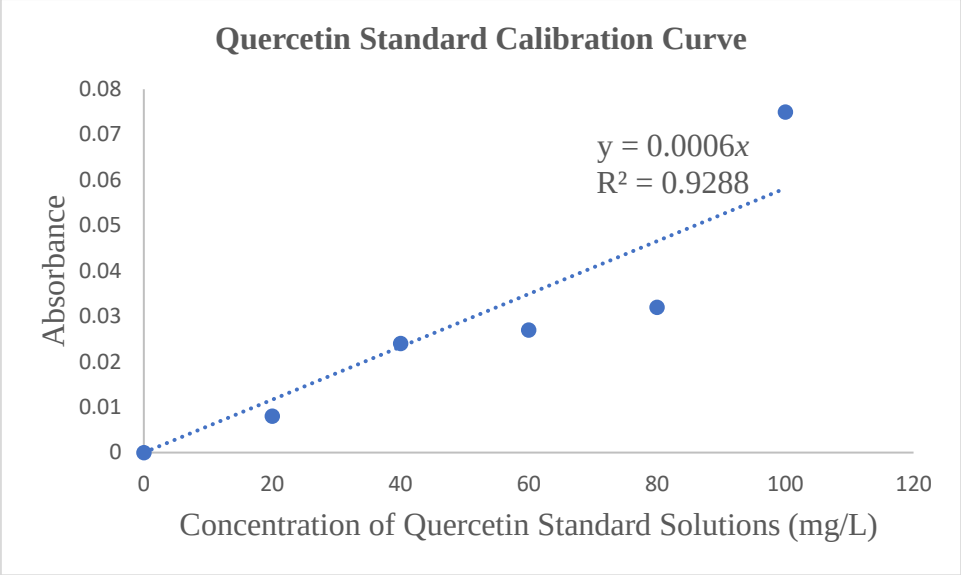

Figure 4.2: Relationship between absorbance values and concentration of quercetin standard solutions.

Table 4.4: Total flavonoid contents of control, palm oil and coconut oil treated brown rice samples cooked by three different cooking methods.

Cooking Methods	Total Flavonoid Content (mg QUE/100 g DW)		
	Control	**Palm Oil**	**Coconut Oil**
I	48.44 ± 13.86^{cy}	160.94 ± 15.63^{cx}	142.19 ± 19.35^{ax}
II	84.38 ± 16.54^{bz}	210.94 ± 17.95^{bx}	143.75 ± 18.40^{ay}
III	120.31 ± 10.67^{az}	273.44 ± 17.95^{ax}	151.56 ± 10.67^{ay}

Presentation of data was in the form of mean ± standard deviation (n = 4).
$^{a–c}$: Significant difference among data within column with different superscripts based on Tukey's HSD test ($p < 0.05$).
$^{x–z}$: Significant difference among data within row with different superscripts based on Tukey's HSD test ($p < 0.05$).

4.5 DPPH Free Radical Scavenging Activity

Figure 4.3 showed the increasing percentage of DPPH free radical scavenging activity with increasing concentrations (5 mg/mL, 10 mg/mL, 15 mg/mL, 20 mg/mL, 25 mg/mL) of pure ascorbic acid and extracts of control, palm oil and coconut oil treated brown rice cooked by three different cooking methods. IC_{50} values of pure ascorbic acid and sample extracts which were calculated through the linear regression analysis were tabulated in Table 4.5. In cooking method I, there was a significant ($p < 0.05$) decrease in the IC_{50} value of palm oil treated brown rice. In cooking method II, IC_{50} value of control was significantly higher than the oil-treated brown rice. In cooking method III, IC_{50} values of cooked brown rice were insignificantly different with each other. For the control samples, higher IC_{50} values were noticed in cooking methods I and II, while cooking method III with lower IC_{50} value was reflected. For the brown rice cooked with palm oil and coconut oil, an insignificant difference ($p > 0.05$) was observed in the IC_{50} values between the three cooking methods.

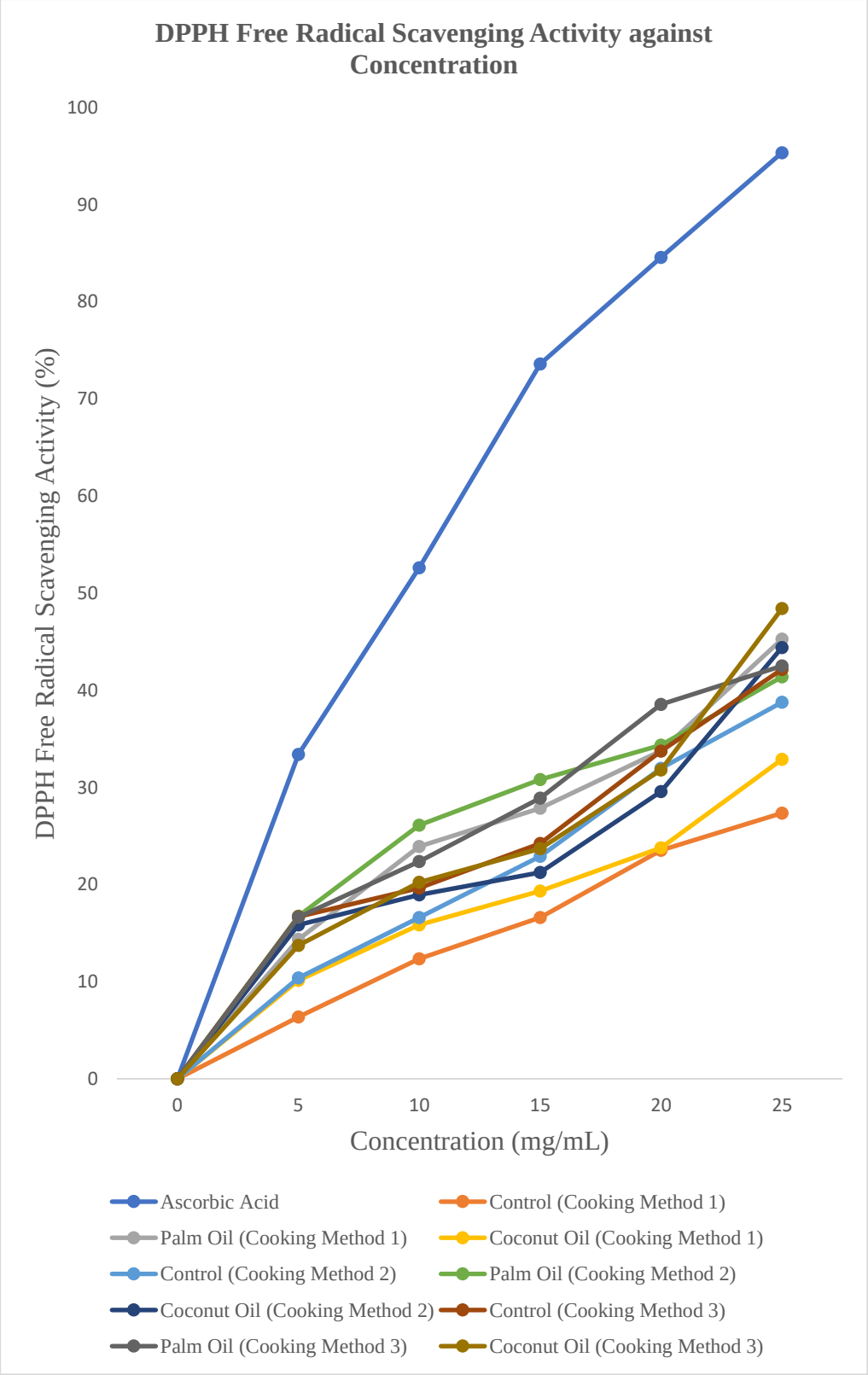

Figure 4.3: Percentage of DPPH free radical scavenging activity against concentrations of pure ascorbic acid and sample extracts of cooked brown rice.

Table 4.5: IC_{50} values of control, palm oil and coconut oil treated brown rice samples cooked by three different cooking methods.

		IC_{50} (mg/mL)		
Pure Acids	**Ascorbic**	11.71 ± 0.00		
Cooking Methods		**Control**	**Palm Oil**	**Coconut Oil**
I		44.55 ± 5.05^{ax*}	27.25 ± 2.33^{ay*}	40.18 ± 9.98^{ax*}
II		39.41 ± 2.62^{ax*}	28.05 ± 5.65^{ay*}	30.26 ± 3.18^{ay*}
III		29.73 ± 5.38^{bx*}	27.00 ± 3.16^{ax*}	28.44 ± 4.89^{ax*}

Presentation of data was in the form of mean ± standard deviation (n = 4).
$^{a–b}$: Significant difference among data within column with different superscripts based on Tukey's HSD test ($p < 0.05$).
$^{x–y}$: Significant difference among data within row with different superscripts based on Tukey's HSD test ($p < 0.05$).
Means with superscript of asterisk (*) were significantly different with pure ascorbic acid based on independent sample T-test.

4.6 Reducing Power Activity

Increasing percentage of ferric reducing power activity with increasing concentrations (5 mg/mL, 10 mg/mL, 15 mg/mL, 20 mg/mL, 25 mg/mL) of pure ascorbic acid and extracts of brown rice cooked by three different cooking methods was illustrated in Figure 4.4. Table 4.5 presented the EC_{50} values of pure ascorbic acid and sample extracts that were calculated through the linear regression analysis. In cooking methods I and II, EC_{50} values of control were significantly higher than the oil treated brown rice. Cooking method III did not significantly affect the EC_{50} values of cooked brown rice samples. For the control and brown rice cooked with palm oil and coconut oil, EC_{50} values in all the three cooking methods were comparable with each other.

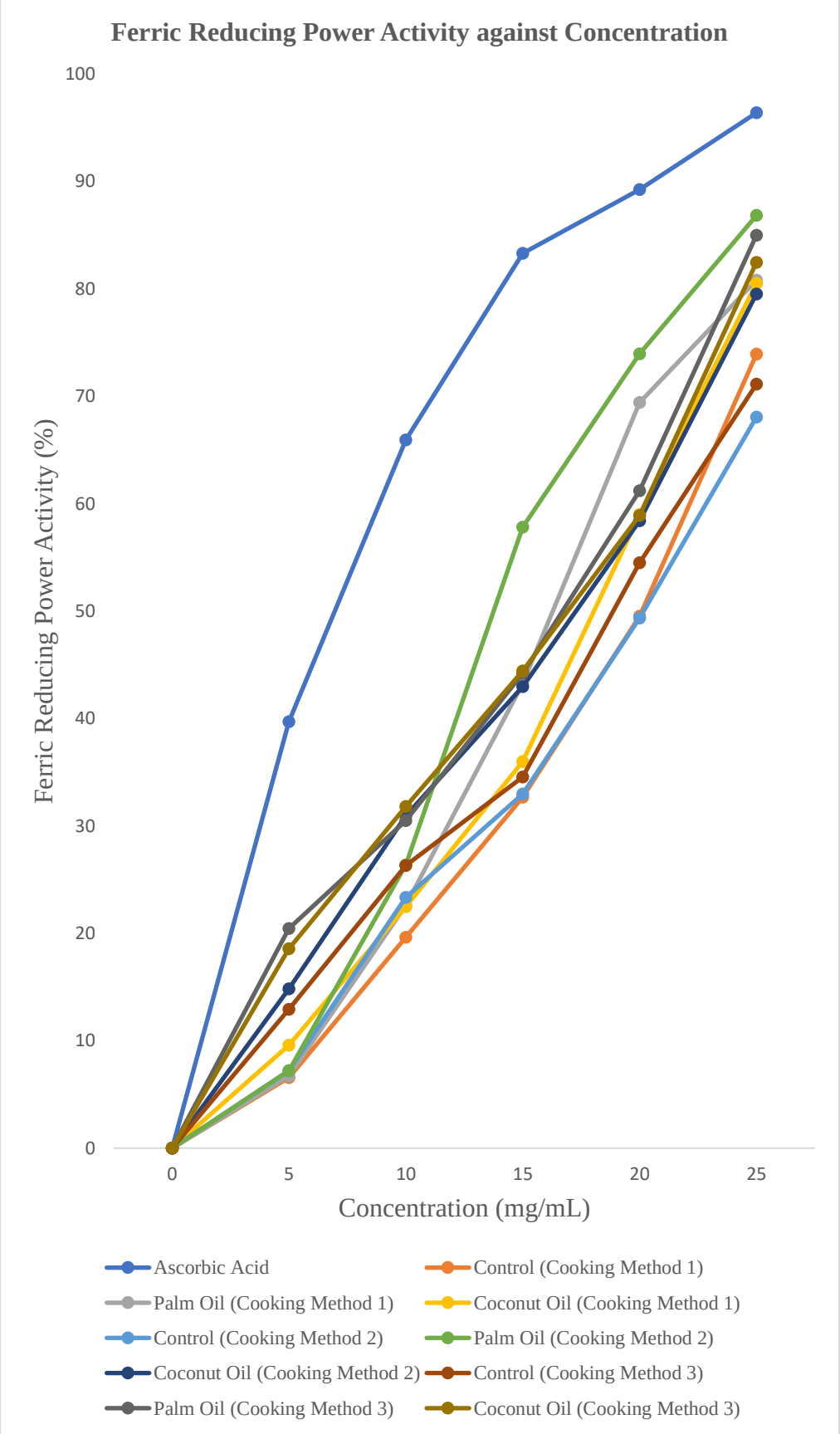

Figure 4.4: Percentage of ferric reducing power activity against concentrations of pure ascorbic acid and sample extracts of cooked brown rice.

Table 4.6: EC_{50} values of control, palm oil and coconut oil treated brown rice samples cooked by three different cooking methods.

		EC_{50} (mg/mL)		
Pure Acids	**Ascorbic**	10.91 ± 0.14		
Cooking Methods		**Control**	**Palm Oil**	**Coconut Oil**
I		$20.04 \pm 1.71^{ax*}$	$16.05 \pm 1.66^{ay*}$	$17.22 \pm 1.08^{ay*}$
II		$20.15 \pm 2.48^{ax*}$	$14.37 \pm 1.41^{ay*}$	$16.48 \pm 0.48^{ay*}$
III		$18.56 \pm 1.08^{ax*}$	$15.59 \pm 0.82^{ax*}$	$16.56 \pm 2.53^{ax*}$

Presentation of data was in the form of mean ± standard deviation (n = 4).
[a]: Insignificant difference among data within column with same superscripts based on Tukey's HSD test ($p > 0.05$).
[x–y]: Significant difference among data within row with different superscripts based on Tukey's HSD test ($p < 0.05$).
Means with superscript of asterisk (*) were significantly different with pure ascorbic acid based on independent sample T-test.

4.7 Correlation Analysis

Correlation coefficients between the tested parameters were tabulated in Table 4.7. There was an insignificant ($p > 0.05$) positive, moderate correlation between TPC and TFC ($r = 0.51$). A significant ($p < 0.05$), strong positive correlation was also shown between IC_{50} and EC_{50} values ($r = 0.78$). Positive correlation demonstrated an increasing trend of TPC with increasing TFC. IC_{50} values also increased together with EC_{50} values. On the other hand, TPC was negatively and moderately correlated with IC_{50} values ($r = -0.49$). Lastly, there was a significant ($p < 0.05$), strong negative correlation between TPC and EC_{50} ($r = -0.71$), between TFC and IC_{50} values ($r = -0.75$), and between TFC and EC_{50}

values (r = -0.86). In terms of negative correlation, IC_{50} and EC_{50} values decreased with increasing TPC and TFC.

Table 4.7: Correlation analysis between total phenolic contents (TPC), total flavonoid contents (TFC), IC_{50} and EC_{50} values.

Parameter	**Correlation coefficient (r)**			
	TPC	**TFC**	**IC_{50}**	**EC_{50}**
TPC	1	0.51	-0.49	-0.71*
TFC	0.51	1	-0.75*	-0.86*
IC_{50}	-0.49	-0.75*	1	0.78*
EC_{50}	-0.71*	-0.86*	0.78*	1

Values with superscript of asterisk (*) were statistically significant ($p < 0.05$).

CHAPTER 5

DISCUSSION

5.1 Color

The presence of yellow and red pigments such as carotenoids and anthocyanins including cyanidin-3-glucoside (451.9 mg/100 g) and peonidin-3-glucoside (42.7 mg/100 g) rendered the bran layer of raw brown rice its brownish-red color (Noh and Zik, 2002). During frying, enzymatic reaction occurred due to the action of oxido-reductase enzymes (Syafutri, et al., 2016). For instance, polyphenol oxidase in the bran layer of brown rice oxidized the phenolic compounds in brown rice, resulting in the enzymatic discoloration of cooked brown rice (Jiang, et al., 2016). However, for the control and oil-treated brown rice, an insignificant difference ($p > 0.05$) was observed in the L* and b* values between the three cooking methods (Table 4.1). Hence, it may be deduced that the additional step of frying for 1 minute in cooking methods I and III did not mark a difference with cooking method II involving only steaming. A 1-minute frying process would probably not increase the rate of enzymatic browning of cooked brown rice (Garber, et al., 2011).

Since the color of the brown rice was governed by the pigments present in the bran layers, the effects of cooking such as steaming and frying on the pigments may lead to the color change of cooked brown rice. During frying and steaming of brown rice, the yellow and red pigments in the outer and inner bran layers diffused into the endosperm (Lamberts, et al., 2006). According to a* and b*

values of control and brown rice cooked with palm oil and coconut oil, it was demonstrated that there was an insignificant difference ($p > 0.05$) in the a* and b* values of cooked brown rice samples between the cooking methods, respectively (Table 4.1). This phenomenon may be supported by the findings of Pal, et al. (2019). The authors stated that mild steaming using boiling water (44 minutes) and frying of less than 5 minutes would not significantly affect the yellowness and redness of cooked brown rice due to the lesser inward diffusion of the yellow and red pigments during the cooking processes. Nevertheless, there was no valid explanation on the effect of cooking on the yellowness and redness of cooked brown rice yet.

In terms of the oil-treated brown rice, due to the treatment of raw brown rice with cooking oils prior to steaming, the added palm and coconut oils tended to act as protective coatings on the surface of rice grains to minimize the leaching of amylopectin and the coating of the cooked rice kernels with leached amylopectin during the steaming process. As a result of the light reflected from the surface of the lipid-coated cooked rice kernels, the lightness of oil-treated brown rice was improved (Olkkonen and Brainard, 2010). Hence, this coincided with the observation showing a significant difference ($p > 0.05$) between the L* values of the control and oil-treated brown rice in cooking method I, and between the L* values of the control and brown rice cooked with palm oil in cooking method II (Table 4.1). The reason behind the insignificant difference ($p > 0.05$) between the L* values of oil and non-oil treated brown rice in the cooking method III could be most probably because the raw brown rice was steamed in the filtered water without the addition of palm and coconut oils as in

the cooking methods I and II. Therefore, during steaming, deposition of leached amylopectin as a viscous layer on the surface of rice kernels may reduce the reflection of light from the cooked rice grains (Raut, et al., 2011; Yang, et al., 2016). Frying the steamed brown rice with the cooking oils for 1 minute after steaming may not be sufficient to induce an impact of the color pigments in cooking oils on the lightness of brown rice (Garber, et al., 2011).

5.2 Texture

Difference between the texture such as hardness and stickiness of raw and cooked brown rice relied on the principle of starch gelatinization. Amylose and amylopectin constituted 20.5% and 79.5% of starch in brown rice, respectively, whose starch granules were in semi-crystalline form (Farooq, et al., 2021). When brown rice was steamed in the presence of water, the applied heat cleaved the glycosidic linkages between the molecules of amylose and amylopectin, as well as their double helix structures. Water molecules diffused into the starch granules through the unbranched amylose to initiate the formation of hydrogen bonds between the water and starch granules (Jackson, 2003). Water absorption tended to continue until the gelatinization temperature of brown rice of 85°C was reached, upon which the irreversible swelling and burst of starch granules occurred, disrupting the crystalline structures of starch granules (Adi, et al., 2020). During the process of starch gelatinization, low molecular weight amylose (31.84%) and short-chain amylopectin (64.29%) leached into the water where the brown rice was steamed in, decreasing the hardness and increasing the stickiness of cooked rice (Yang, et al., 2016). This was proven by the study

performed by Jain and his colleagues (2012) which demonstrated a decrease in the amylose content of steamed brown rice from 25.99% to 17.35% after steaming for 40 minutes. Hence, based on the hardness values of cooked brown rice (Table 4.2), it was shown that the control reported significantly lower hardness values than the oil-treated brown rice in cooking methods I and II.

Xu, et al. (2018) stated that the texture of brown rice was governed by the amylose and amylopectin contents which varied based on the variety of brown rice. In this study, the same type of unpolished brown rice was used in all the cooking methods, with constant variables such as the water-to-rice ratio and duration of steaming. Hence, it was inferred that similar amylose content of cooked brown rice would be resulted. Therefore, for the control samples, the hardness values were insignificantly different between the three cooking methods (Table 4.2). By focusing on the control samples, although cooking method I and III involved frying, the effect of frying was dispensable as Garber, et al. (2011) claimed that frying time of 1 minute was incapable of imposing effect on the texture of cooked brown rice.

With the short chain amylopectin making up the highest percentage of starch components in the leached starch, the final amylose and amylopectin contents of cooked brown rice were determined by the amounts of the starch components leached out during cooking (Thuengtung and Ogawa, 2019). Oils or lipids acted as the inhibitor of starch gelatinization. Owing to the hydrophobic properties of oil, the hydration of starch granules may be retarded due to the coatings of starch

granules by the lipids (Adi, et al., 2020). In this case, starch gelatinization was delayed. Therefore, in the cooking methods I and II, the hardness values of palm oil and coconut oil treated brown rice in the respective range of 18515.83 ± 186.66 g to 18833.95 ± 157.96 g and 17982.53 ± 130.81 g to 18953.53 ± 111.94 g were significantly higher than their control samples in the corresponding methods, 13947.98 ± 145.23 g for cooking method I and 12260.30 ± 159.26 g for cooking method II (Table 4.2). The increased hardness of oil-treated cooked rice was supported by Bi, et al. (2018) who mentioned that the addition of vegetable oil during rice cooking led to a significant ($p < 0.05$) increase in the firmness of cooked rice. However, in cooking method III, the hardness of oil and non-oil treated brown rice were insignificantly different with each other (Table 4.2). This may be caused by the disrupted crystalline structure of starch granules that already happened during steaming (without oil) due to starch gelatinization, thus minimizing the effect of oil on texture of cooked rice during frying that was done after steaming (Liu, et al., 2009).

In terms of the stickiness of cooked brown rice, the cooked rice grains that were coated by the short-chain amylopectin leached into the rice water as a viscous layer may be evidenced by the appearance of sticky cooked rice (Yang, et al., 2016). Due to the inversely proportional relationship between hardness and stickiness, harder cooked rice was found to be less sticky (Cameron and Wang, 2005). This was consistent with the readings of stickiness of the cooked brown rice in the cooking methods I and II, whereby significantly lower stickiness values were observed in the oil-treated brown rice with significantly higher values of hardness (Table 4.2).

5.3 Total Phenolic Content (TPC)

Phenolic compounds or polyphenols found in bran layers and germ of brown rice include phenolic acids. They are further classified into hydroxycinnamic acids and hydroxybenzoic acids with total concentrations of 76.87 mg GAE (gallic acid equivalent)/100 g DW (Rosnaini and Abdullah, 2016). During steaming, hydrophilic phenolic compounds may leach into the cooking water used (Thuengtung and Ogawa, 2019). Hence, according to the total phenolic contents of cooked brown rice (Table 4.3), for control sample, total phenolic content of control in cooking method II was significantly lower than in cooking methods I and III. This was agreed by the study done by Chmiel, et al. (2017). The authors concluded that steaming using boiling water for 30 minutes with water to rice ratio of 2:1 decreased the total phenolic contents of both polished and unpolished brown rice by 18–28%. Besides, reduced total phenolic contents of pigmented rice by 33.5% after 40-minute steaming was claimed by Fracassetti, et al. (2020). A loss of total phenolic contents of cooked rice by 16%–57% and 27%–38% was also stated by Min, McClung and Chen (2014) and Zaupa, et al. (2015), respectively.

According to the study of Chmiel, et al. (2017), the average total phenolic content of brown rice cooked by steaming for 30 minutes was 49 mg GAE/100 g DW. On the other hand, the total phenolic content of control sample that was steamed for 40 minutes in cooking method II in this study showed 23.13 mg GAE/100 g DW (Table 4.3). The disparity of the results could be explained by the difference in the duration of steaming as Bhawamai, et al. (2016) mentioned

that less antioxidative compounds would be retained in the case of longer cooking time. This may be due to the lixiviation of more hydrophilic phenolics into the cooking water used during longer duration of steaming (Thuengtung and Ogawa, 2019). This statement was supported by the study of Siah, et al. (2014), in which a significant ($p < 0.05$) decrease in the total phenolic contents was caused by the liberation of 40%–68% of water-soluble phenolics into the water used to soak and cook fava beans. Different total phenolic contents of cooked brown rice observed may also be influenced by the variety of brown rice used (Chmiel, et al., 2017).

The loss of phenolic compounds after cooking was also rendered by the thermal instability of phenolic compounds (Liazid, et al., 2007). Phenolic compounds consisted of hydroxyl groups that made them to be antioxidative with the ability of scavenging free radicals. During the thermal process at high temperature, intermolecular bonds of hydroxyl groups were cleaved, thus breaking the phenolic compounds into smaller molecules (low molecular weights) with decreased antioxidant activity due to the loss of hydroxyl groups (Sun, Bai and Zhuang, 2012). Moreover, the hydrophobic, free phenolic acids may react with macromolecules in brown rice. For instance, reaction between the phenolic acids and leached amylose, and protein respectively resulted in the synthesis of inclusion compounds and other compounds that could not be extracted due to the non-hydrolysable covalent bonds (Ozdal, Capanoglu and Altay, 2013; Surh and Koh, 2014). Thermal effect of cooking process may also lead to the amylolytic and proteolytic distortion of cell wall structure where the phenolic acids such as ferulic acids and p-coumaric acids were bound to via ester or ether

bonds. Consequently, the liberated bound phenolic acids were in free form and subjected to thermal decomposition (Shahidi and Yeo, 2016). The rate of thermal degradation of phenolic compounds via oxidation was increased during frying with the exposure to moisture and oxygen (Min, McClung and Chen, 2014). Hence, it was estimated that the total phenolic contents of cooked brown rice in cooking methods I and III involving frying would be lower than in the cooking method II. However, it was found that a contradictory result was obtained, in which the average total phenolic contents of control in cooking method III and I were higher than in cooking method II (Table 4.3). This may be associated with the short frying time of 1 minute that was not able to initiate the oxidative degradation of phenolic compounds (Alide, Wangila and Kiprop, 2020). On the other hand, there was another argument portraying that frying at 160°C for 3 minutes may give rise to a significant ($p < 0.05$) rise in p-hydroxybenzoic acids and gallic acids contents (Sun, Bai and Zhuang, 2012).

Based on the research of Teh, et al. (2021), it was documented that palm oil had total phenolic content of 3.26 mg GAE/kg as compared to coconut oil with total phenolic content of 1.74 mg GAE/kg. In this context, the highest total phenolic contents of palm oil treated brown rice in cooking methods I and II was within expectation (Table 4.3). It was hypothesized that the higher the total phenolic contents of used vegetable oil were, the higher the total phenolic contents of the cooked brown rice would be, due to the compensation for the phenolic compounds lost from the brown rice during cooking (Sahin, et al., 2020). However, in cooking method III, despite the addition of vegetable oils during cooking, there was an insignificant difference ($p > 0.05$) between the total

phenolic contents of non-oil and oil-treated rice (Table 4.3). This may be due to the different sequence of steps in adding cooking oils. As compared to cooking method I and II where the cooking oils were added to raw brown rice, in cooking method III, palm oil and coconut oil were added to steamed brown rice before frying. Hence, distorted crystalline orders of steamed brown rice may inhibit the diffusion of phytochemicals in cooking oils into the destroyed structure of steamed rice during the subsequent frying step (Liu, et al., 2009).

5.4 Total Flavonoid Content (TFC)

Among polyphenols, examples of flavonoids found in the grains of brown rice, particularly in the bran layers are quercetin and kaempferol (Ravichanthiran, et al., 2018). It was stated that the brown rice bran had a concentration of total flavonoids of up to 788.21 mg QUE (quercetin equivalent)/100 g DW (Ghasemzadeh, et al., 2018). Based on the results of total flavonoid contents of cooked brown rice (Table 4.4), it was summarized that the control samples had the significantly lower total flavonoid contents in all the three cooking methods. This was in tandem with the study justifying the loss of total flavonoids by 12%–61% after steaming for 40 minutes (Min, McClung and Chen, 2014). Ti and his co-workers (2015) also reported that cooking for 20 minutes reduced total flavonoids contents of brown rice by 72.8%.

Thermal degradation of flavonoids during cooking was mainly responsible for the reduction in the total flavonoid contents of cooked brown rice due to the sensitivity of flavonoid compounds to thermal treatment (Ismail, Marjan and

Foong, 2004). Glycosylation of flavonoids contributed to the high bioavailability of the phytochemicals in foods (Slámová, Kapešová, and Valentová, 2018). Fuleki and Silva (2003) indicated that heat treatment may induce the depolymerization of flavonoids in dimers and oligomers into monomers by hydrolyzing the C-glycosidic bonds joined between the molecules. Besides, inhibition of biosynthesis pathway of flavonoids at temperature of above 75°C was denoted by Zhang, et al. (2019). Thermal breakdown of flavonoids may also occur during cooking (Nayeem, et al., 2021). Water-soluble flavonoids such as anthocyanins (3.09 mg CGE/100 g), for instance, peonidin-3-*O*-glucoside, cyanidin-3-*O*-glucoside, and cyanidin-3-*O*-galactoside in the aleurone layers of brown rice were thermally destroyed in two stages (Mackon, et al., 2021; Ravichanthiran, et al., 2018). The glycosidic linkages of anthocyanins were degraded following the disruption of pyrylium rings during the thermal cooking treatment, causing the conversion into chalcone structure or α-diketones. Upon the removal of benzene ring, the chalcone structure was then broken down into coumarin glucoside derivative (Patras, et al., 2010). Hence, a loss of the anthocyanin contents of cooked pigmented rice by 50% was declared, suggesting the heat lability of anthocyanins (Kechinski, et al., 2010).

In contrast to the results of total phenolic contents (Table 4.3), for control and palm oil treated brown rice, their total flavonoid contents in cooking method I that involved stir-frying prior to steaming were lower, while the higher total flavonoid contents were shown in cooking method III (frying after steaming) (Table 4.4). Stir-frying is considered as one of the dry heating methods (Dreeling, Allen and Butler, 2000). Bener, et al. (2013) clarified that more

flavonoids would be decomposed in the cooking method using dry heat as compared to the wet-heat cooking method such as steaming. However, there was an opposing statement by Huarte, et al. (2021) who claimed that frying for 10–30 minutes may increase the total flavonoid contents by 3.65 folds, but flavonoid compounds were completely degraded after frying time of 30 minutes. Alide, Wangila and Kiprop (2020), on the other hand, stated that no significant effect ($p > 0.05$) would be observed in the total flavonoid contents with a cooking time of less than 5 minutes. Therefore, since frying of brown rice was only carried out for 1 minute in this study, the effect of frying on the total flavonoid contents of brown rice required further justification.

The comparison between the total flavonoid contents of oil-treated brown rice and control was in concordance with the results of total phenolic contents due to the similar trend in the total flavonoid contents of palm and coconut oils. The study of Teh, et al. (2021) found that palm oil contained more flavonoids with total flavonoid content of 4.36 mg RE (rutin equivalent)/kg which was double the total flavonoid content of coconut oil (2.49 mg RE/kg). Hence, in cooking methods II and III, a significant difference ($p < 0.05$) between control and brown rice cooked with palm oil and coconut oil was in agreement (Table 4.4). In cooking method I, although the total flavonoid contents of palm oil and coconut oil treated brown rice were insignificantly different with each other, their total flavonoid contents were still significantly higher than that of the control (Table 4.4). This suggested the positive effect of vegetable oil addition on the antioxidant contents of rice during cooking.

5.5 DPPH Free Radical Scavenging Activity

DPPH radical scavenging assay was applied to determine antioxidant activity of samples. Compounds with antioxidative properties in the rice extracts were hydrogen-donating agents. They neutralized the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical in the methanolic solution of DPPH to 2,2-diphenyl-1-picryl-hydrazine which was a stable, diamagnetic non-radical. Hence, a change in the color of solution from purple to yellow was observed (Gangwar, et al., 2014). The intensity of yellow or purple color corresponded to the antioxidant activity of the compounds in the rice extracts. The antioxidant activity of the extracts was represented by the term, IC_{50} which was the concentration of extracts necessary for the quench of 50% of free radicals (Jadid, et al., 2017). Hence, extract with high free radical scavenging activity would have a low IC_{50} value.

In the study of evaluating antioxidant activity of cooked brown rice, pure ascorbic acid was used as a reference standard due to its role as a primary antioxidant with two enolic hydroxyl groups (2,3-enediol) that readily scavenged free radicals (Njus, et al., 2020). This was to compare the DPPH radical scavenging activity of sample extracts of cooked brown rice with the pure ascorbic acid. Since the pure ascorbic acid solution was used without the addition of other extract, antioxidant compound in pure ascorbic acid solution was responsible for the DPPH radical scavenging activity of the solution without interferences from other impurities (Munteanu and Apetrei, 2021). Therefore, the IC_{50} value of pure ascorbic acid was significantly different from those of the extracts of cooked brown rice (Table 4.5 and Appendix J). The

significantly lowest IC_{50} value of pure ascorbic acid signified its potent antioxidant activity as compared to the sample extracts (Table 4.5).

In the measurement of the free radical scavenging activity of the extracts and pure ascorbic acid solutions, a concentration dependent manner was illustrated (Pavithra and Vadivukkarasi, 2015). At high concentration of the sample solutions, the increased concentration of the antioxidant compounds were able to scavenge more DPPH free radicals, leading to a higher degree of discoloration of the solutions from purple to yellow color (Pereira, et al., 2011). Hence, this was in concordance with the increased percentage of DPPH radical scavenging activity with increasing concentrations of solutions (Figure 4.3).

The results of IC_{50} values of the extracts of cooked brown rice were corresponded to their total phenolic and flavonoid contents in each cooking method. In cooking method I, palm oil treated brown rice with significantly higher total phenolic content displayed an IC_{50} value which was significantly lower than that of the coconut oil treated brown rice and control (Table 4.5). On the other hand, in cooking method II, the oil-treated brown rice samples also recorded a significantly lower IC_{50} value, representing their higher DPPH free radical scavenging activity due to their significantly higher total phenolic and flavonoid contents (Table 4.5). The antioxidant power of phenolic and flavonoid compounds was attributed to the chemical structures of the molecules. For instance, stabilization of free radicals by the phenolic rings (Woo, et al., 2018). The negative moderate correlation between total phenolic contents and IC_{50} (r

= -0.49) and negative strong correlation between total flavonoid contents and IC_{50} (r = -0.75) further corroborated the contribution of phenolics and flavonoids to high DPPH activity of brown rice with low IC_{50} (Table 4.7). Apart from the phenolics and flavonoids, antioxidant activity may also be presented by phytosterol and β-glucan in brown rice (Sen, Chakraborty and Kalita, 2020).

In terms of the control brown rice sample, as compared to the oil-treated brown rice, a significantly higher IC_{50} value was observed in cooking method II (Table 4.5). The decrease in the antioxidant activity of the control coincided with the study of Nayeem, et al. (2021). The authors reflected that thermal treatment or cooking reduced the DPPH activity of pigmented rice by 10%. A loss of antioxidant activity of cooked brown rice by 14%–42% according to DPPH was also observed after a steaming time of 30 minutes (Chmiel, et al., 2017). The study of Shahidi, et al. (2016) also demonstrated a significant decrease ($p < 0.05$) in the DPPH activity of cooked rice. However, the percentage of decrease of the DPPH radical scavenging activity was not specified. Reduced antioxidant activity was tally with the loss of total phenolic and flavonoid contents of cooked brown rice which was mainly due to the thermal chemical reaction of the antioxidative compounds (Saikia, et al., 2012).

5.6 Reducing Power Activity

In reducing power assay, antioxidant activity of antioxidative compounds in rice extracts was suggested by their electron-donating ability of reducing Fe^{3+} in potassium ferricyanide to Fe^{2+}. Synthesis of Fe^{2+}-Fe^{3+} complex resulted in a

color change of solution from colorless to blue (Jayanthi and Lalitha, 2011). Antioxidant activity of extracts was assessed by the term EC_{50}. High antioxidant activity was supported by low EC_{50} value as only low concentration of extract was required for 50% of antioxidative effect (Chen, Bertin and Froldi, 2013).

The antioxidative properties of pure ascorbic acid were known for its role of reducing agent that readily acted as an electron donor (Akbari, et al., 2016). Therefore, similar to DPPH assay, pure ascorbic acid was used as positive control to compare with the reducing power activity of cooked brown rice extracts. In the reducing power assay, EC_{50} value of 10.91 ± 0.14 mg/mL of pure ascorbic acid was significantly lower than that of the extracts of cooked brown rice (Table 4.6 and Appendix L). This denoted the purity of the antioxidant compounds in pure ascorbic acid which was associated with its high electron-donating ability as compared to the rice extracts (Munteanu and Apetrei, 2021).

Intensity of blue color of solutions depended on the concentrations of Fe^{2+}-Fe^{3+} colored complexes formed which was directly related to the concentrations of antioxidant compounds present in the solutions of sample extracts and pure ascorbic acid. Hence, a dose dependent phenomenon was also demonstrated in the reducing power assay, as in the DPPH assay (Pavithra and Vadivukkarasi, 2015). In concentrated solutions, the presence of more antioxidants such as phenolic and flavonoid ions resulted in the generation of more Fe^{2+}-Fe^{3+} colored complexes due to the transfer of electrons from the antioxidants to the ferric ions (Bhalodia, et al., 2013). Therefore, this led to a more concentrated blue

color with an increasing absorbance, which contrasted with the decreasing absorbance of the solutions in DPPH assay caused by discoloration. If this was the case, in reducing power assay, the greater difference between the absorbance of negative control (without sample) and the sample solutions gave rise to a higher percentage of ferric reducing power activity calculated. This reflected the increased antioxidant activity as the solution concentrations increased (Figure 4.4).

EC_{50} values was strongly and negatively correlated with total phenolic contents ($r = -0.71$) and total flavonoid contents ($r= -0.86$) of rice extracts (Table 4.7). Therefore, in cooking method I, brown rice cooked with palm oil and coconut oil had higher antioxidant activity with significantly lower EC_{50} values which was substantiated with their significantly higher total flavonoid contents than the control (Table 4.6). On the other hand, in cooking method II, significantly higher total phenolic and flavonoid contents of palm oil and coconut oil treated brown rice contributed to their significantly lower EC_{50} values (Table 4.6).

According to the results of EC_{50} values of cooked brown rice (Table 4.6), control recorded the significantly higher values of EC_{50} in cooking methods I and II than the oil-treated brown rice. The reason behind the decreased reducing power activity of control was emphasized on the thermal breakdown of antioxidants such as phenolics and flavonoids that changed their status as antioxidants, which was also shown in the DPPH assay (Min, McClung and Chen, 2014). Thermal decomposition of chemical structures of flavonoids was

also related to the loss of flavonoids in cooked rice (Kmiecik, et al., 2015). The study of Min, McClung and Chen (2014) agreed with the loss of antioxidant activity of brown rice after cooking with a statement that brown rice that was steamed for 40 minutes experienced a 38%–51% decrease in the ability of chelating the ferric ions. Meanwhile, Nayeem, et al. (2021) declared that cooking reduced the antioxidant activity of brown rice by 60%. This was in accordance with the study of Xu and Chang (2009) which mentioned a loss of reducing power ability by 69%–72% after thermal treatment. A significant ($p < 0.05$) decrease in the reducing power activity after thermal cooking such as boiling, steaming, and frying was also revealed (Sun, Bai and Zhuang, 2012).

5.7 Correlation Analysis

Owing to the classification of flavonoids under the phenolic compounds, total phenolic contents tended to increase with total flavonoid contents (Iqbal, Salim and Lim, 2015). This was evidenced by a positive moderate correlation between TPC and TFC ($r = 0.51$) (Table 4.7). A strong positive correlation between IC_{50} and EC_{50} values ($r = 0.78$) was also within expectation since both IC_{50} and EC_{50} values represented the antioxidant activity of samples (Table 4.7). Besides, negative correlation between TPC and TFC with IC_{50} and EC_{50} values implied the attribution of DPPH and reducing power activity of cooked brown rice to the total phenolic and flavonoid contents, in which low IC_{50} and EC_{50} values indicated high DPPH and reducing power activity, respectively. This was in tandem with the findings of Mir, et al. (2015) and Owolabi, et al. (2018) which

revealed a strong positive correlation between total phenolic and flavonoid contents with the DPPH and reducing power activity.

5.8 Limitations and Future Study Recommendations

In the present study, high standard deviation was observed in some of the data in the color and texture analysis and antioxidant assays. Hence, it was suggested to double the number of replicates in the future study to improve the precision of the results. Besides, in this study, antioxidant contents of cooked brown rice were determined in terms of total phenolic and flavonoid contents. However, the types of phenolic and flavonoid compounds in cooked brown rice were unknown. Hence, high performance liquid chromatography (HPLC) was recommended, to obtain an antioxidant profile of the cooked brown rice, as well as the concentration of each identified antioxidant compound. In this case, specific types of antioxidants that would be affected by thermal cooking could be determined, as different antioxidants would have different degree of thermal stability due to their varied chemical structures.

Lastly, for a more comprehensive study of antioxidant activity of cooked brown rice, antioxidant activity assays should not be limited to DPPH radical scavenging activity and reducing power assays. Antioxidant assays capable of studying the antioxidant capacity such as $ABTS^{\bullet+}$ radical scavenging and hydroxyl radical scavenging activity assays should also be conducted. In this way, the results of antioxidant activity of cooked brown rice observed in all the assays could be compared with each other for the correlation analysis.

CHAPTER 6

CONCLUSION

Brown rice was cooked using three different cooking methods involving steaming and/or frying with/without palm and coconut oils. Cooking significantly decreased lightness (L*) of control in cooking methods I and II due to enzymatic discoloration. However, cooking methods did not significantly affect redness (a*) and yellowness (b*) of rice. Despite the intense yellow color of palm oil, there was an insignificant difference ($p > 0.05$) between L* values of palm oil and coconut oil treated rice in cooking methods I and III. Besides, cooking decreased hardness and increased stickiness of control in cooking methods I and II due to starch gelatinization. In cooking methods I and II, oil-treated rice showed higher hardness and lower stickiness due to delayed starch hydration by lipids which acted as inhibitor of starch gelatinization. Moreover, cooking significantly reduced TPC and TFC of control in cooking methods I and II. This was attributed to the leaching of hydrophilic phytochemicals into cooking water during steaming, and thermal decomposition of phytonutrients during frying that decreased their DPPH radical scavenging and reducing power activities which were expressed in terms of IC_{50} and EC_{50}, respectively. However, due to high antioxidant contents in palm oil, TPC and TFC of palm oil treated rice were higher than the coconut oil treated rice and control in cooking methods I and II. This corresponded to its higher antioxidant activity with lower IC_{50} and EC_{50} in the cooking methods. Hence, cooking brown rice with palm oil using cooking methods I and II was suggested to be the preferred cooking methods due to the increased TPC, TFC and antioxidant activities.

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APPENDICES

APPENDIX A

Tukey's HSD test results for L* values in cooking method I

L* Cooking Method I

Tukey B[a]

Group	N	Subset for alpha = 0.05	
		1	2
Control	4	63.0200	
Coconut Oil	4		66.8400
Palm Oil	4		68.3000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for L* values in cooking method II

L* Cooking Method II

Tukey B[a]

Group	N	Subset for alpha = 0.05	
		1	2
Control	4	65.2025	
Coconut Oil	4	66.4650	
Palm Oil	4		69.6125

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for L* values in cooking method III

L* Cooking Method III

Tukey B[a]

Group	N	Subset for alpha = 0.05
		1
Control	4	65.2850
Coconut Oil	4	66.1975
Palm Oil	4	66.9475

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for L* values of control

L* Control

Tukey B[a]

Group	N	Subset for alpha = 0.05
		1
Cooking Method I	4	63.0200
Cooking Method II	4	65.2025
Cooking Method III	4	65.2850

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for L* values of palm oil treated brown rice

L* Palm Oil

Tukey B[a]

Group	N	Subset for alpha = 0.05
		1
Cooking Method III	4	66.9475
Cooking Method I	4	68.3000
Cooking Method II	4	69.6125

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for L* values of coconut oil treated brown rice

L* Coconut Oil

Tukey B[a]

Group	N	Subset for alpha = 0.05
		1
Cooking Method III	4	66.1975
Cooking Method II	4	66.4650
Cooking Method I	4	66.8400

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

APPENDIX B

Tukey's HSD test results for a* values in cooking method I

a* Cooking Method I

Tukey B[a]

Group	N	Subset for alpha = 0.05
		1
Control	4	.2275
Coconut Oil	4	.2700
Palm Oil	4	.4150

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for a* values in cooking method II

a* Cooking Method II

Tukey B[a]

Group	N	Subset for alpha = 0.05
		1
Coconut Oil	4	.2850
Palm Oil	4	.3050
Control	4	.6825

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for a* values in cooking method III

a* Cooking Method III

Tukey B[a]

Group	N	Subset for alpha = 0.05
		1
Control	4	.3650
Palm Oil	4	.5200
Coconut Oil	4	.7625

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for a* values of control

a* Control

Tukey B[a]

Group	N	Subset for alpha = 0.05
		1
Cooking Method I	4	.2275
Cooking Method III	4	.3650
Cooking Method II	4	.6825

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for a* values of palm oil treated brown rice

a* Palm Oil

Tukey B[a]

Group	N	Subset for alpha = 0.05
		1
Cooking Method II	4	.3050
Cooking Method I	4	.4150
Cooking Method III	4	.5200

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for a* values of coconut oil treated brown rice

a* Coconut Oil

Tukey B[a]

Group	N	Subset for alpha = 0.05
		1
Cooking Method I	4	.2700
Cooking Method II	4	.2850
Cooking Method III	4	.7625

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

APPENDIX C

Tukey's HSD test results for b* values in cooking method I

b* Cooking Method I

Tukey B[a]

Group	N	Subset for alpha = 0.05
		1
Coconut Oil	4	11.9225
Palm Oil	4	12.2275
Control	4	12.9400

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for b* values in cooking method II

b* Cooking Method II

Tukey B[a]

Group	N	Subset for alpha = 0.05
		1
Coconut Oil	4	13.9625
Palm Oil	4	14.5750
Control	4	14.9200

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for b* values in cooking method III

b* Cooking Method III

Tukey B[a]

Group	N	Subset for alpha = 0.05
		1
Palm Oil	4	13.1250
Control	4	14.3975
Coconut Oil	4	14.7675

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for b* values of control

b* Control

Tukey B[a]

Group	N	Subset for alpha = 0.05
		1
Cooking Method I	4	12.9400
Cooking Method III	4	14.3975
Cooking Method II	4	14.9200

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for b* values of palm oil treated brown rice

b* Palm Oil

Tukey B[a]

Group	N	Subset for alpha = 0.05
		1
Cooking Method I	4	12.2275
Cooking Method III	4	13.1250
Cooking Method II	4	14.5750

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for b* values of coconut oil treated brown rice

b* Coconut Oil

Tukey B[a]

Group	N	Subset for alpha = 0.05
		1
Cooking Method I	4	11.9225
Cooking Method II	4	13.9625
Cooking Method III	4	14.7675

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

APPENDIX D

Independent sample T-test results for total color differences in cooking method I

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Cooking Method I	Equal variances assumed	2.295	.181	1.531	6	.177	.89198	.58251	-.53337	2.31732
	Equal variances not assumed			1.531	3.597	.208	.89198	.58251	-.79938	2.58333

Independent sample T-test results for total color differences in cooking method II

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Cooking Method II	Equal variances assumed	6.265	.046	.552	6	.601	.60245	1.09114	-2.06747	3.27237
	Equal variances not assumed			.552	3.081	.618	.60245	1.09114	-2.81909	4.02399

Independent sample T-test results for total color differences in cooking method III

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Cooking Method III	Equal variances assumed	.035	.859	.324	6	.757	.37720	1.16290	-2.46831	3.22271
	Equal variances not assumed			.324	5.779	.757	.37720	1.16290	-2.49492	3.24932

Tukey's HSD test results for total color differences of palm oil treated brown rice

Color Diff Palm Oil

Tukey B[a]

Group	N	Subset for alpha = 0.05
		1
Cooking Method III	4	3.785475
Cooking Method II	4	4.928450
Cooking Method I	4	5.528700

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for total color differences of coconut oil treated brown rice

Color Diff Coconut Oil

Tukey B[a]

Group	N	Subset for alpha = 0.05
		1
Cooking Method III	4	3.408275
Cooking Method II	4	4.326000
Cooking Method I	4	4.636725

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

APPENDIX E

Tukey's HSD test results for hardness in cooking method I

Hardness Cooking Method I

Tukey B[a]

Group	N	Subset for alpha = 0.05	
		1	2
Control	4	13947.98200	
Coconut Oil	4		18515.83075
Palm Oil	4		18833.94975

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for hardness in cooking method II

Hardness Cooking Method II

Tukey B[a]

Group	N	Subset for alpha = 0.05	
		1	2
Control	4	12260.30075	
Coconut Oil	4		17982.52775
Palm Oil	4		18953.64325

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for hardness in cooking method III

Hardness Cooking Method III

Tukey B[a]

Group	N	Subset for alpha = 0.05
		1
Control	4	13302.27075
Coconut Oil	4	13561.38450
Palm Oil	4	13869.37100

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for hardness of control

Hardness Control

Tukey B[a]

Group	N	Subset for alpha = 0.05
		1
Cooking Method II	4	12260.30075
Cooking Method III	4	13302.27075
Cooking Method I	4	13947.98200

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for hardness of palm oil treated brown rice

Hardness Palm Oil

Tukey B[a]

Group	N	Subset for alpha = 0.05	
		1	2
Cooking Method III	4	13869.37100	
Cooking Method I	4		18833.94975
Cooking Method II	4		18953.64325

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for hardness of coconut oil treated brown rice

Hardness Coconut Oil

Tukey B[a]

Group	N	Subset for alpha = 0.05	
		1	2
Cooking Method III	4	13561.38450	
Cooking Method II	4		17982.52775
Cooking Method I	4		18515.83075

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

APPENDIX F

Tukey's HSD test results for stickiness in cooking method I

Stickiness Cooking Method I

Tukey B[a]

Group	N	Subset for alpha = 0.05	
		1	2
Coconut Oil	4	2.31625	
Palm Oil	4	2.45350	
Control	4		3.47850

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for stickiness in cooking method II

Stickiness Cooking Method II

Tukey B[a]

Group	N	Subset for alpha = 0.05	
		1	2
Palm Oil	4	2.42975	
Coconut Oil	4	2.61150	
Control	4		3.53175

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for stickiness in cooking method III

Stickiness Cooking Method III

Tukey B[a]

Group	N	Subset for alpha = 0.05
		1
Coconut Oil	4	3.11000
Palm Oil	4	3.28250
Control	4	3.31850

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for stickiness of control

Stickiness Control

Tukey B[a]

Group	N	Subset for alpha = 0.05
		1
Cooking Method III	4	3.31850
Cooking Method I	4	3.47850
Cooking Method II	4	3.53175

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for stickiness of palm oil treated brown rice

Stickiness Palm Oil

Tukey B[a]

Group	N	Subset for alpha = 0.05	
		1	2
Cooking Method II	4	2.42975	
Cooking Method I	4	2.45350	
Cooking Method III	4		3.28250

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for stickiness of coconut oil treated brown rice

Stickiness Coconut Oil

Tukey B[a]

Group	N	Subset for alpha = 0.05	
		1	2
Cooking Method I	4	2.31625	
Cooking Method II	4	2.61150	
Cooking Method III	4		3.11000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

APPENDIX G

Tukey's HSD test results for TPC in cooking method I

TPC Cooking Method I

Tukey B[a]

Group	N	Subset for alpha = 0.05	
		1	2
Control	4	37.5000	
Coconut Oil	4	49.3750	
Palm Oil	4		68.4375

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for TPC in cooking method II

TPC Cooking Method II

Tukey B[a]

Group	N	Subset for alpha = 0.05		
		1	2	3
Control	4	23.1250		
Coconut Oil	4		41.5625	
Palm Oil	4			52.1875

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for TPC in cooking method III

TPC Cooking Method III

Tukey B[a]

Group	N	Subset for alpha = 0.05
		1
Control	4	38.7500
Coconut Oil	4	39.3750
Palm Oil	4	47.5000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for TPC of control

TPC Control

Tukey B[a]

Group	N	Subset for alpha = 0.05	
		1	2
Cooking Method II	4	23.1250	
Cooking Method I	4		37.5000
Cooking Method III	4		38.7500

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's test results for TPC of palm oil treated brown rice

TPC Palm Oil

Tukey B[a]

Group	N	Subset for alpha = 0.05	
		1	2
Cooking Method III	4	47.5000	
Cooking Method II	4	52.1875	
Cooking Method I	4		68.4375

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's test results for TPC of coconut oil treated brown rice

TPC Coconut Oil

Tukey B[a]

Group	N	Subset for alpha = 0.05
		1
Cooking Method III	4	39.3750
Cooking Method II	4	41.5625
Cooking Method I	4	49.3750

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

APPENDIX H

Tukey's HSD test results for TFC in cooking method I

TFC Cooking Method I

Tukey B[a]

Group	N	Subset for alpha = 0.05	
		1	2
Control	4	48.4375	
Coconut Oil	4		142.1875
Palm Oil	4		160.9375

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for TFC in cooking method II

TFC Cooking Method II

Tukey B[a]

Group	N	Subset for alpha = 0.05		
		1	2	3
Control	4	84.3750		
Coconut Oil	4		143.7500	
Palm Oil	4			210.9375

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for TFC in cooking method III

TFC Cooking Method III

Tukey B[a]

Group	N	Subset for alpha = 0.05		
		1	2	3
Control	4	120.3125		
Coconut Oil	4		151.5625	
Palm Oil	4			273.4375

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for TFC of control

TFC Control

Tukey B[a]

Group	N	Subset for alpha = 0.05		
		1	2	3
Cooking Method I	4	48.4375		
Cooking Method II	4		84.3750	
Cooking Method III	4			120.3125

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for TFC of palm oil treated brown rice

TFC Palm Oil

Tukey B[a]

Group	N	Subset for alpha = 0.05		
		1	2	3
Cooking Method I	4	160.9375		
Cooking Method II	4		210.9375	
Cooking Method III	4			273.4375

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for TFC of coconut oil treated brown rice

TFC Coconut Oil

Tukey B[a]

Group	N	Subset for alpha = 0.05
		1
Cooking Method I	4	142.1875
Cooking Method II	4	143.7500
Cooking Method III	4	151.5625

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

APPENDIX I

Tukey's HSD test results for IC_{50} values in cooking method I

IC50 Cooking Method I

Tukey B[a]

Group	N	Subset for alpha = 0.05	
		1	2
Palm Oil	4	27.24750	
Coconut Oil	4		40.18050
Control	4		44.55100

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for IC_{50} values in cooking method II

IC50 Cooking Method II

Tukey B[a]

Group	N	Subset for alpha = 0.05	
		1	2
Palm Oil	4	28.04725	
Coconut Oil	4	30.26275	
Control	4		39.41400

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for IC_{50} values in cooking method III

IC50 Cooking Method III

Tukey B[a]

Group	N	Subset for alpha = 0.05
		1
Palm Oil	4	26.99950
Coconut Oil	4	28.43550
Control	4	29.72500

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for IC_{50} values of control

IC50 Control

Tukey B[a]

Group	N	Subset for alpha = 0.05	
		1	2
Cooking Method III	4	29.72500	
Cooking Method II	4		39.41400
Cooking Method I	4		44.55100

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for IC_{50} values of palm oil treated brown rice

IC50 Palm Oil

Tukey B[a]

Group	N	Subset for alpha = 0.05
		1
Cooking Method III	4	26.99950
Cooking Method I	4	27.24750
Cooking Method II	4	28.04725

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for IC_{50} values of coconut oil treated brown rice

IC50 Coconut Oil

Tukey B[a]

Group	N	Subset for alpha = 0.05
		1
Cooking Method III	4	28.43550
Cooking Method II	4	30.26275
Cooking Method I	4	40.18050

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

APPENDIX J

Independent sample T-test results for comparison between IC_{50} values of control and pure ascorbic acid in cooking method I

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
IC50	Equal variances assumed	4.099	.113	-8.680	4	<.001	-32.84500	3.78384	-43.35062	-22.33938
	Equal variances not assumed			-13.020	3.000	<.001	-32.84500	2.52256	-40.87290	-24.81710

Independent sample T-test results for comparison between IC_{50} values of palm oil treated brown rice and pure ascorbic acid in cooking method I

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
IC50	Equal variances assumed	3.357	.141	-8.908	4	<.001	-15.54150	1.74472	-20.38563	-10.69737
	Equal variances not assumed			-13.362	3.000	<.001	-15.54150	1.16315	-19.24314	-11.83986

Independent sample T-test results for comparison between IC_{50} values of coconut oil treated brown rice and pure ascorbic acid in cooking method I

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
IC50	Equal variances assumed	19.929	.011	-3.797	4	.019	-28.47450	7.49909	-49.29532	-7.65368
	Equal variances not assumed			-5.696	3.000	.011	-28.47450	4.99940	-44.38480	-12.56420

Independent sample T-test results for comparison between IC_{50} values of control and pure ascorbic acid in cooking method II

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
IC50	Equal variances assumed	3.598	.131	-14.079	4	<.001	-27.70800	1.96810	-33.17233	-22.24367
	Equal variances not assumed			-21.118	3.000	<.001	-27.70800	1.31207	-31.88357	-23.53243

Independent sample T-test results for comparison between IC_{50} values of palm oil treated brown rice and pure ascorbic acid in cooking method II

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
IC50	Equal variances assumed	136.749	<.001	-3.854	4	.018	-16.34125	4.23955	-28.11214	-4.57036
	Equal variances not assumed			-5.782	3.000	.010	-16.34125	2.82637	-25.33601	-7.34649

Independent sample T-test results for comparison between IC_{50} values of coconut oil treated brown rice and pure ascorbic acid in cooking method II

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
IC50	Equal variances assumed	3.204	.148	-7.773	4	.001	-18.55675	2.38722	-25.18474	-11.92876
	Equal variances not assumed			-11.660	3.000	.001	-18.55675	1.59148	-23.62154	-13.49196

Independent sample T-test results for comparison between IC_{50} values of control and pure ascorbic acid in cooking method III

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
IC50	Equal variances assumed	28.095	.006	-4.466	4	.011	-18.01900	4.03449	-29.22053	-6.81747
	Equal variances not assumed			-6.699	3.000	.007	-18.01900	2.68966	-26.57869	-9.45931

Independent sample T-test results for comparison between IC_{50} values of palm oil treated brown rice and pure ascorbic acid in cooking method III

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
IC50	Equal variances assumed	3.522	.134	-6.449	4	.003	-15.29350	2.37143	-21.87765	-8.70935
	Equal variances not assumed			-9.674	3.000	.002	-15.29350	1.58096	-20.32479	-10.26221

Independent sample T-test results for comparison between IC_{50} values of coconut oil treated brown rice and pure ascorbic acid in cooking method III

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
IC50	Equal variances assumed	38.184	.003	-4.562	4	.010	-16.72950	3.66714	-26.91111	-6.54789
	Equal variances not assumed			-6.843	3.000	.006	-16.72950	2.44476	-24.50981	-8.94919

APPENDIX K

Tukey's HSD test results for EC_{50} values in cooking method I

EC50 Cooking Method I

Tukey B[a]

Group	N	Subset for alpha = 0.05	
		1	2
Palm Oil	4	16.04750	
Coconut Oil	4	17.21975	
Control	4		20.04200

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for EC_{50} values in cooking method II

EC50 Cooking Method II

Tukey B[a]

Group	N	Subset for alpha = 0.05	
		1	2
Palm Oil	4	14.36625	
Coconut Oil	4	16.47675	
Control	4		20.14875

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for EC_{50} values in cooking method III

EC50 Cooking Method III

Tukey B[a]

Group	N	Subset for alpha = 0.05
		1
Palm Oil	4	15.58675
Coconut Oil	4	16.55775
Control	4	18.55875

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for EC_{50} values of control

EC50 Control

Tukey B[a]

Group	N	Subset for alpha = 0.05
		1
Cooking Method III	4	18.55875
Cooking Method I	4	20.04200
Cooking Method II	4	20.14875

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for EC_{50} values of palm oil treated brown rice

EC50 Palm Oil

Tukey B[a]

Group	N	Subset for alpha = 0.05
		1
Cooking Method II	4	14.36625
Cooking Method III	4	15.58675
Cooking Method I	4	16.04750

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tukey's HSD test results for EC_{50} values of coconut oil treated brown rice

EC50 Coconut Oil

Tukey B[a]

Group	N	Subset for alpha = 0.05
		1
Cooking Method II	4	16.47675
Cooking Method III	4	16.55775
Cooking Method I	4	17.21975

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

APPENDIX L

Independent sample T-test results for comparison between EC_{50} values of control and pure ascorbic acid in cooking method I

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
EC50	Equal variances assumed	3.984	.117	-7.124	4	.002	-9.13300	1.28206	-12.69257	-5.57343
	Equal variances not assumed			-10.624	3.083	.002	-9.13300	.85967	-11.82776	-6.43824

Independent sample T-test results for comparison between EC_{50} values of palm oil treated brown rice and pure ascorbic acid in cooking method I

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
EC50	Equal variances assumed	5.240	.084	-4.114	4	.015	-5.13850	1.24906	-8.60644	-1.67056
	Equal variances not assumed			-6.133	3.087	.008	-5.13850	.83779	-7.76266	-2.51434

Independent sample T-test results for comparison between EC_{50} values of coconut oil treated brown rice and pure ascorbic acid in cooking method I

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
EC50	Equal variances assumed	2.337	.201	-7.759	4	.001	-6.31075	.81339	-8.56908	-4.05242
	Equal variances not assumed			-11.473	3.201	.001	-6.31075	.55004	-8.00057	-4.62093

Independent sample T-test results for comparison between EC_{50} values of control and pure ascorbic acid in cooking method II

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
EC50	Equal variances assumed	3.958	.118	-4.961	4	.008	-9.23975	1.86230	-14.41032	-4.06918
	Equal variances not assumed			-7.422	3.039	.005	-9.23975	1.24495	-13.17279	-5.30671

Independent sample T-test results for comparison between EC_{50} values of palm oil treated brown rice and pure ascorbic acid in cooking method II

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
EC50	Equal variances assumed	3.256	.145	-3.268	4	.031	-3.45725	1.05778	-6.39411	-.52039
	Equal variances not assumed			-4.861	3.121	.015	-3.45725	.71119	-5.67180	-1.24270

Independent sample T-test results for comparison between EC_{50} values of coconut oil treated brown rice and pure ascorbic acid in cooking method II

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
EC50	Equal variances assumed	19.130	.012	-15.096	4	<.001	-5.56775	.36882	-6.59175	-4.54375
	Equal variances not assumed			-21.203	3.789	<.001	-5.56775	.26260	-6.31309	-4.82241

Independent sample T-test results for comparison between EC_{50} values of control and pure ascorbic acid in cooking method III

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
EC50	Equal variances assumed	2.215	.211	-9.391	4	<.001	-7.64975	.81461	-9.91147	-5.38803
	Equal variances not assumed			-13.887	3.201	<.001	-7.64975	.55084	-9.34219	-5.95731

Independent sample T-test results for comparison between EC_{50} values of palm oil treated brown rice and pure ascorbic acid in cooking method III

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
EC50	Equal variances assumed	2.843	.167	-7.543	4	.002	-4.67775	.62018	-6.39963	-2.95587
	Equal variances not assumed			-11.043	3.336	<.001	-4.67775	.42361	-5.95217	-3.40333

Independent sample T-test results for comparison between EC_{50} values of coconut oil treated brown rice and pure ascorbic acid in cooking method III

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
EC50	Equal variances assumed	295.170	<.001	-2.974	4	.041	-5.64875	1.89954	-10.92273	-.37477
	Equal variances not assumed			-4.449	3.038	.021	-5.64875	1.26971	-9.66113	-1.63637

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