Evaluation of Physicochemical and Antioxidant Activity of *Rosa damascena* and *Osmanthus fragrans* Infused Rambutan Vinegar

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DECLARATION

I hereby declare that the work embodied in this report is the result of the original research and has not been submitted for a higher degree to any Universities or institutions.

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Date : 21st February 2022

I certify that the report of this final year project entitled "**Evaluation of Physicochemical and Antioxidant Activity of *Rosa damascena* and *Osmanthus fragrans* infused Rambutan Vinegar**" by **Loh Huey Qi**, matric number **F18A0052** has been examined and all the correction recommended by supervisor have been done for the degree of Bachelor of Applied Science (Product Development Technology) with Honours, Faculty of Agro Based Industry, University Malaysia Kelantan.

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Ciri-Ciri Fizikokimia dan Aktiviti Antioksida Cuka Rambutan yang diinfusi dengan *Rosa damascena* dan *Osmanthus fragrans*

ABSTRAK

Kelebihan antioksida cuka Rambutan *(Nephelium lappaceum)* tidak diketahui secara meluas dan pasaran cuka juga menunjukkan sifat fizikal yang tidak menarik seperti warna dan baunya mangakibatkan cuka gagal menarik perhatian pengguna. Bunga kering yang boleh dimakan seperti Rose *(Rosa damascena)* dan Osmanthus *(Osmanthus fragrans)* diinfusi dalam Cuka Rambutan masih belum dikeluarkan di pasaran Malaysia dan kelebihannya tidak diketahui secara meluas. Ujian DPPH digunakan untuk menentukan aktiviti antioksida, kaedah Folin-Ciocalteua digunakan untuk menentukan jumlah kandungan fenolik, kaedah kolorimetrik aluminium klorida digunakan untuk menentukan jumlah kandungan flavonoid dan kaedah pembezaan pH AOAC digunakan untuk menentukan pigmen antosianin bagi Cuka Rambutan yang diinfusi dengan bunga. Sifat fizikokimia seperti analisis warna, pH, jumlah pepejal larut (TSS), kandungan asid asetik dan aktiviti antioksida Cuka Rambutan yang diinfusi bunga Rose (RVR) dan Cuka Rambutan yang diinfusi bunga Osmanthus (RVO) menunjukkan perbezaan jika dibandingkan dengan Cuka Rambutan (RV). Aktiviti antioksida RVR dan RVO menunjukkan peningkatan yang ketara berbanding RV. Aktiviti antioksida RV(3 g Rose) pada minggu ke-2 (68.37%) adalah lebih tinggi daripada RV(1 g Osmanthus) pada minggu ke-2 (29.92%). Jumlah kandungan fenolik, jumlah kandungan flavonoid dan pigmen antosianin RVR dan RVO menunjukkan peningkatan yang ketara berbanding RV. RV(3 g Rose) pada minggu ke-2 menunjukkan jumlah kandungan fenolik tertinggi (152.29 GAE µg/mL) dan pigmen antosianin tertinggi (1.89 C3G mg/L) manakala RV(1 g Osmanthus) pada minggu ke-2 menunjukkan jumlah kandungan flavonoid tertinggi (1.79 QE mg/mL). Sebagai rumusan, jumlah bunga yang diinfusi dalam RV dan masa infusi yang terbaik untuk RVR ialah RV(3 g Rose) pada minggu ke-2 dan RVO ialah RV(1 g Osmanthus) pada minggu ke-2.

Kata kunci: cuka yang diinfusi, warna, sifat fizikokimia, sifat antioksida

Evaluation of Physicochemical and Antioxidant Activity of *Rosa damascena* and *Osmanthus fragrans* infused Rambutan Vinegar

ABSTRACT

The antioxidant benefits of Rambutan (*Nephelium lappaceum*) vinegar are not widely known and also the vinegar in the market shows unappealing physical properties such as its colour and smell, resultantly, fails to attract consumers' attention. Dried edible flower such as Rose (*Rosa damascena)* and Osmanthus (*Osmanthus fragrans)* infused vinegar have yet to be released in the Malaysian market and its benefits is not widely known. DPPH assay was used to determine the antioxidant activity, Folin-Ciocalteua's method was used to determine total phenolic content, aluminium chloride colorimetric method was used to determine total flavonoid content and AOAC pH differential method was used to measure anthocyanin pigment of flower infused Rambutan Vinegar (RV). The physicochemical properties such as the colour analysis, pH, total soluble solid (TSS), acetic acid content and antioxidant activity of Rose infused Rambutan Vinegar (RVR) and Osmanthus infused Rambutan Vinegar (RVO) show differences when compared to RV. Antioxidant activity of RVR and RVO shows significance increase compared to RV. The antioxidant activity of RV(3 g Rose) at Week 2 (68.37%) is higher than RV(1 g Osmanthus) at Week 2 (29.92%). The total phenolic content, total flavonoid content and anthocyanin pigment of RVR and RVO shows significant increase compared to RV. RV(3 g Rose) at Week 2 shows the highest total phenolic content (152.29 GAE µg/mL) and highest anthocyanin pigment (1.89 C3G mg/L) whereas RV(1 g Osmanthus) at Week 2 shows the highest total flavonoid content (1.79 QE mg/mL). To summarise, the best concentration of edible flowers infused in RV and time of infusion for RVR is RV(3 g Rose) at Week 2 and RVO is (RV(1 g Osmanthus) at Week 2.

Keywords: infused vinegar, colour, physicochemical properties, antioxidant properties

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LIST OF ABBREVIATIONS

AAB	Acetic acid bacteria
$AlCl_3$	Aluminium chloride
ANOVA	Analysis of Variance
AOAC	Association of Official Agricultural Chemists
C3G	Cyanidin-3-Glucoside
CH_3CO_2Na	Sodium acetate
CH_3COOH	Acetic acid
C_2H_5OH	Ethanol
DF	Dilution factor
DPPH	2,2-diphenyl-1-picrylhydrazyl
FCR	Folin-Ciocalteu reagent
GAE	Gallic acid equivalent
H_2O	Water
HCl	Hydrochloric acid
KCl	Potassium chloride
M	Molarity
MW	Molecular weight
$NaNO_3$	Sodium nitrate
NaOH	Sodium hydroxide
O_2	Oxygen
ORAC	Oxygen Radical Absorbance Capacity
QE	Quercetin Equivalent
ROS	Reactive Oxygen Species
RV	Rambutan Vinegar

RVR	Rose infused Rambutan Vinegar
RVO	Osmanthus infused Rambutan Vinegar
TSS	Total Soluble Solid

LIST OF UNITS

ºBx	Degree Brix
mg/L	Milligram per litre
µg/mL	Microgram per millilitre
g	Gram
L	litres
M	Molar
mL	Millilitre
mg	Milligram
nm	Nanometre
v/w	Volume per weight

LIST OF SYMBOLS

ºC	Degree Celsius
%	Percent
µ	Micro
λmax	Maximum wavelength
ε	Molar extinction coefficient
H_0	Null hypothesis
H_1	Alternate hypothesis
±	Plus minus
<	Less than

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Vinegar is an acidic liquid which was discovered since 3000 B.C. It was made by accident when the fermentation of wine took too long which resulted in the birth of vinegar which originated from a French word '*vinaigre'*, which means 'sour wine'. Naturally fermented vinegar is usually fermented from fruits due to its high sugar content which allows the two-stage fermentation to take place. The first fermentation involves the breaking down of sugars from fruit juice into alcohol (anaerobic fermentation) by yeasts and then second fermentation involve the oxidation of alcohol to acetic acid (aerobic fermentation) by *Acetobactor* and *Gluconobacter* (Budak *et al*., 2014). Vinegar has many uses such its function to add flavour to food, preserve food and it can also be added into cleaning routine as a cleaning agent. There are various types of natural vinegar in the market which include Malt vinegar that is produced from malting barley, Wine vinegar that is produced from red or white wine, Balsamic vinegar that is produced from aging of concentrated grapes juice and Fruit vinegar that is produced from pure fruit juices

including apple, blueberry, pear and others (Bamforth and Cook, 2019). Based on a study, with the purpose to decrease the post-harvest losses of fruits in developing countries, the excess fruits are used to produce vinegar which can then be used to preserve food at the same time reduce wastage of fruits (Adebayo-Oyetoro *et al*., 2017). Since Malaysia is a developing country with abundant production of tropical fruits, the post-harvest losses of tropical fruits have been an issue faced by Malaysian government. With this in mind, a research on vinegar production from Rambutan (*Nephelium lappaceum*) and Dokong (*Lansium domesticum*) was conducted to address the post-harvest losses of Malaysian tropical fruits, Rambutan (*Nephelium lappaceum*) and Dokong (*Lansium domesticum*) (Mokhtar *et al*., 2016).

With the recent rise of health consciousness and increased education level among consumers, natural vinegar such as fruit vinegar is much desired in the market. According to a survey conducted among the residents living in Kelantan and Kuala Lumpur on customer acceptance of Dokong (*Lansium domesticum*) & Rambutan (*Nephelium lappaceum*), it was stated that the consumers favoured Natural Vinegar in comparison with Artificial Vinegar (Mokhtar *et al.,* 2016). Moreover, the nutritional benefits of Rambutan Vinegar are almost at par with Apple Cider Vinegar by having higher protein, potassium, calcium, magnesium and manganese contents (Mokhtar *et al*., 2016). In addition, Rambutan Vinegar has a higher pH value (less acidic) compared to the commercialized Apple Cider Vinegar in which more consumers would appreciate it. However, Rambutan Vinegar is not as popular as the commercialized Apple Cider Vinegar due to its low exposure among consumers. Hence, aromatization of Rambutan Vinegar with fruits, herbs and spices as a way to introduce Rambutan Vinegar to consumers. The vinegar can be aromatized by steeping the fruits, herbs and spices in the

vinegar when the two-stage fermentation process of vinegar is complete (Plessi, 2003). Aromatized vinegars add unique and extraordinary tastes to food and drinks.

Edible flowers such as chrysanthemum, calendula, nasturtium, rose and lavender have been used in the preparation of food as a source of colouring, flavouring, fragrance and decoration on food and drinks since centuries ago (Mlcek and Rop, 2011). Edible flowers have high content of phytochemicals such as flavonoids, anthocyanins and phenols (Loizzo *et al.*, 2015). Furthermore, edible flowers have high antioxidant activities which can help to neutralize the effect of free radicals such as reactive oxygen species (ROS) which result in the occurrence of oxidative stress that will damage the biomembranes and affect the DNA molecules, proteins or enzymes (Fu and Mao, 2018; Sharma *et al*., 2012). For example, Rose (*Rosa damascena*) is one of the edible flowers with the highest antioxidant activity and the highest total phenolic content (Hu *et al*., 2019). Edible flowers such as Osmanthus (*Osmanthus fragrans)* apart from being used as flavouring ingredient, it is also used to prevent degenerative diseases because the abundant amounts of polyphenols and antioxidants which helps in regulating the plasma antioxidant status in healthy people (Li *et al*., 2013).

Therefore, in this research, in order to introduce Rambutan Vinegar to the market, new ways to consume Rambutan Vinegar is proposed through infusing Rambutan Vinegar with Rose (*Rosa damascena*) and Osmanthus (*Osmanthus fragrans*). The infusion of Rambutan Vinegar with flowers will not only improve the colour and taste of the vinegar but also its antioxidants level and nutritional values. The interactions between the flower with the Rambutan Vinegar were determined through physicochemical analysis and antioxidant analysis of the infused vinegar.

1.2 Problem Statement

Vinegar consists of many health benefits such as improving the process of digestion, stimulating appetite, has high content of antioxidants, energy recovery effects, helps to lower cholesterol level and also control the blood pressure (Chin *et al*, 2016; Chou *et al.*, 2015). However, the antioxidant benefit of vinegar is not widely known and also the vinegar in the market shows unappealing physical properties such as its colour and smell, resultantly, fails to attract consumers' attention.

Besides, there is limited research based on infused vinegar as most of the infusion technology is mostly applied on oils. For example, infusion of herbs and spices such as truffle in extra virgin olive oil is very popular in the market because the oils will have the flavour of the truffle which can be used as a food condiment such as salad dressings and also during cooking to increase the flavour of the dish (Gambacorta *et al*., 2007).

Furthermore, edible flower infused vinegar has yet to be released in the Malaysian market. For this reason, with the high antioxidants, nice aroma and colour of the edible flowers, they are ideal ingredients to be infused into the Rambutan Vinegar to create a new product and hence, as a new way to consume Rambutan Vinegar and also a new line of cosmetic products could be produced.

1.3 Hypothesis

H_o : There is no significance difference between the concentration of edible flower and time of infusion on colour of flower infused Rambutan Vinegar.

H_1 : There is significance difference between the concentration of edible flower and time of infusion on colour of flower infused Rambutan Vinegar.

H_o : There is no significance difference between the concentration of edible flower and time of infusion on the physicochemical properties (pH, total soluble solid and acetic acid content) of flower infused Rambutan Vinegar.

H_1 : There is significance difference between the concentration of edible flower and time of infusion on the physicochemical properties (pH, total soluble solid and acetic acid content) of flower infused Rambutan Vinegar.

H_o : There is no significance difference between the concentration of edible flower and time of infusion on the antioxidant activity of flower infused Rambutan Vinegar.

H_1 : There is significance difference between the concentration of edible flower and time of infusion on the antioxidant activity of flower infused Rambutan Vinegar.

H_o : There is no relationship between the antioxidant activity and time of infusion of the flower infused Rambutan Vinegar.

H_1 : There is relationship between the antioxidant activity and time of infusion of the flower infused Rambutan Vinegar.

H_o : There is no significance difference between the selected edible flower infused Rambutan Vinegar on the total phenolic content, total flavonoid content and anthocyanin pigment of flower infused Rambutan Vinegar.

H_1 : There is significance difference between the selected edible flower infused Rambutan Vinegar on the total phenolic content, total flavonoid content and anthocyanin pigment of flower infused Rambutan Vinegar.

1.4 Objectives

The purpose of this research is to enhance the properties of Rambutan Vinegar by infusing with edible flowers through these objectives :

1. To compare the physicochemical properties (colour, pH, total soluble solid content and acetic acid content) between two flowers infused Rambutan Vinegars in 4 weeks.
2. To determine antioxidant activity of the two flowers infused Rambutan Vinegars.
3. To determine total phenolic content, total flavonoid content and anthocyanin pigment of the two flowers infused Rambutan Vinegars.

1.5 Scope of Study

The scope of this research includes the analysis that were used to determine the interaction between dried edible flowers with the Rambutan Vinegar. This research involves two samples which are two dried edible flowers. The colour, pH, Total Soluble Solid (TSS) content and acetic acid content of the dried edible flowers infused Rambutan Vinegar were tested using physicochemical analysis.

Moreover, several concentrations of dried edible flowers of each sample and also a control which was pure Rambutan Vinegar were set up in this research. All the dried edible flowers infused with Rambutan Vinegar with different concentrations has undergone antioxidant analysis. The samples with the highest antioxidant activity were chosen for the determination of Total Phenolic Content (TPC), Total Flavonoid Content (TFC) and Anthocyanin pigment which allows the study and comparison of the phenolic contents, flavonoids and anthocyanins present in the dried edible flower infused Rambutan Vinegar.

1.6 Significance of Study

Vinegar is usually consumed directly as a beverage, used as a food condiment and to preserve fruits and vegetables due to its health benefits and its properties to enhance the flavour of food (Plessi, 2003). Since Malaysia has abundant tropical fruits, Rambutan Vinegar is produced to counter the rising problem of post-harvest losses (Mokhtar *et al.,* 2016). The nutritional values of Rambutan Vinegar are comparable to the commercialized Apple Cider Vinegar, but not many consumers have the exposure to Rambutan Vinegar.

Moreover, with the strong acidity and sour smell of vinegar, not many people enjoy drinking vinegar despite the health benefits vinegar offered. Therefore, infused vinegar with fruits, herbs and spices is a new way to increase consumers' acceptance in vinegar consumption. Furthermore, infused vinegar not only increased the antioxidants and nutritional values of vinegar but also helped in giving a unique and extraordinary taste to the vinegar.

Considering not many edible flowers infused vinegar is available in the Malaysian market, it is an ideal choice to infuse dried edible flower into the Rambutan Vinegar. This can increase the variation of vinegars in the Malaysian market simultaneously introduce new ways to consume Rambutan Vinegar to consumers. The usage of edible flowers in food preparation is very popular. Edible flowers are usually used for decorative purposes, aroma and colour in beautifying objects including food.

Moreover, infused vinegar not only enhance the colour and taste for food, but it can also be used for personal care and cosmetic purposes. The antimicrobial properties of acetic acid which were present in Rambutan Vinegar was effective against acne causing

bacteria (Mokhtar *et al*., 2020). Since Rose has skin anti-inflammatory properties and Osmanthus has rich amounts of acteoside which inhibit melanogenesis and helps in skin lightening, Rose infused Rambutan Vinegar and Osmanthus infused Rambutan Vinegar can be further developed into a cosmetic product (Tae *et al*., 2018 ; Liu *et al*., 2018).

1.7 Limitation of Study

There are several limitations that serve as a barrier when conducting the research. One of the limitations of this study is the time of sampling of the flower infused Rambutan Vinegar which was only done on weekly basis. Sampling could be done in every 3 days to better analyze the physicochemical and antioxidant properties of flower infused Rambutan Vinegar that changes over time. Moreover, there is only one type of control in this research which was the 5% Rambutan Vinegar which serve as the internal control. External control such as other vinegar could be added to compare the results with the flower infused Rambutan Vinegar.

CHAPTER 2

LITERATURE REVIEW

2.1 Vinegar

Vinegar is an acidic liquid from by-product during the fermentation process of alcohol. The word vinegar originated from French word "*vin*" "*aigre*" which means "sour wine" (Plessi, 2003). Vinegar is produced from double fermentation which involves both anaerobic and aerobic fermentation (Adams, 1999). This is because during anaerobic fermentation, fermentable sugars were converted into ethanol by yeast and during aerobic fermentation, the acetification of ethanol by acetic acid bacteria (*Acetobacter*) takes place (Bhat *et al.*, 2014). Moreover, vinegar can be produced using several methods such as Orleans process (slow method), generator process (fast method) and submerged culture process. The vinegar production industry uses a generator process and submerged culture process to produce more vinegar in a shorter period of time (Sankpal, 2019).

Vinegar is used as a basic seasoning in cooking food, used as a food condiment and to preserve food since centuries ago. Since vinegar also has antimicrobial and

antioxidant properties, it is used as a medicine for physical injuries (Sankpal, 2019). For instance, during World War I, vinegar was used to treat wounds (Mas *et al.*, 2016). Furthermore, vinegar also has other therapeutic properties such as antitumor, anti-obesity, reduces blood sugar level and also lowers the level of bad cholesterol in the body.

2.2 Fruit Vinegar

In general, fruit vinegar is made from microbial fermentation of fruits with high sugar content such as apples, mango, pineapple and grapes. In some cases, in order to decrease fruit waste, fruit peels are used to produce fruit vinegars. For example, sweet orange peels which is regarded as waste that have no value are used to produce fruit vinegar with the aim to substitute cider vinegar (Oguntoyinbo *et al*., 2011). However, not all fruit vinegars are produced through anaerobic and aerobic fermentation. There are fruit vinegars that are produced by infusing fruits or combining fruit juice and sugars into rice vinegar. For instance, in Taiwan, most of the fruit vinegars are produced by aromatizing glutinous rice vinegar with fruits or mixing fruit juice and sugars with glutinous rice vinegar. The fruits are usually steeped or mixed with rice vinegar for 3 months to allow the nutrients, aroma and sweet taste from the fruits to be extracted into the rice vinegar which acts as a solvent (Ou and Chang, 2009). Most manufacturer of fruit vinegars do not produce fruit vinegar from anaerobic and aerobic fermentation because it is very time consuming and costly.

Similar to rice vinegar, fruit vinegar also has many health benefits such as control

blood sugar level due to its anti-glycemic effects and lowers the level of cholesterol. According to Petsiou *et al.* (2014), the triglyceride and very-low-density lipoprotein levels in body decreased by the acetic acid in Apple Cider Vinegar. Apart from its health benefits, fruit vinegar also helps in the process of weight loss because of its high level of antioxidants such as quercetin (Mohamad *et al.*, 2020).

Fruit vinegar has also become more popular compared to carbonated beverages as more consumers are aware of the nutritional values of fruit vinegar (Dai *et al.*, 2018). In the recent years, the vinegar industry has become more refined and diversified because of the rise in demand for 100% natural fruit vinegar (Mo *et al.*, 2013). Hence, 100% natural fruit vinegar such as Apple Cider vinegar, Persimmon vinegar and Strawberry vinegar are produced in other countries and Rambutan and Dokong Vinegar are produced in Malaysia.

2.2.1 Rambutan Vinegar

In Malaysia, there are many famous and delicious tropical fruits such as mangosteen, dokong, pulasan, durian and rambutan. Rambutan (*Nephelium lappaceum*) is a seasonal tropical fruit that grows abundantly in tropical countries such as Thailand, Malaysia and Indonesia. The name of Rambutan originated the word "rambut" which means hairy in Malay-Indonesian language. Rambutan is also known as "hairy litchi" (Morton, 1987). Rambutan is in the shape of oval and has the colour of yellow to orange blush and full red in colour. The Rambutan peel has many hairs and it has a juicy translucent-white flesh. The taste of Rambutan is very sweet (depending on ripeness) with a hint of sour taste and the shape of the Rambutan seed is almost similar to almond (Mahmood *et al*., 2017).

Rambutan has potential to be used in the industry of traditional and modern medicine due to its health benefits. This is because Rambutan has high amounts of antioxidants, phenols and flavonoid in its fruit peel (Mistriyani *et al*., 2018). Rambutan also have antimicrobial, anti-dengue, anti-allergic and anti-diabetic effects (Shahrajabian *et al.*, 2020). Moreover, Rambutan grows in abundance during tropical fruits season to the extent of most landed houses in Malaysia with Rambutan tree will produce Rambutan fruit during this season resulting the issue of Rambutan fruits being dumped and left to rot as a waste. Hence, the solution to this issue is to use the excess Rambutan fruits to produce Rambutan Vinegar.

Rambutan Vinegar is produced from deseeded Rambutan fruits through the method of two-stage fermentation (alcoholic and acetic fermentation). The Rambutan Vinegar has a mild Rambutan taste and has a light yellow colour. Besides, Rambutan

Vinegar nutrition value is comparable to the commercialized Apple Cider Vinegar by having higher protein, potassium, calcium, magnesium and manganese contents (Mokhtar *et al.*, 2016) Moreover, Rambutan Vinegar is produced with the aim of reducing the import of natural vinegar in Malaysia (Mokhtar *et al.*, 2016).

Figure 2.1 Rambutan Vinegar

2.3 Infused Vinegar

Infused vinegar is also known as flavoured or aromatized vinegar. Infused vinegar can be produced by infusing herbs, fruits and flowers into the vinegar. The incorporation of herbs and fruits into vinegar produce vinegar with interesting scent and taste. By infusing herbs and fruits into vinegar also improve the colour, antioxidant content and nutritional value of vinegar. Since some herbs have low concentration of sugar which is less than the required sugar content for fermentation to take place, the flavour of the herbs can be obtained by infusing them into vinegar. Moreover, fruit vinegar requires a long period of time for fermentation to take place, hence, as an alternative to obtain fruit flavour in vinegar, fruits such as pomegranate and berries are infused into vinegar (Tan, 2005; Hailu *et al.*, 2012; Hemke *et al.*, 2020). Flavoured vinegar has an increased in popularity among consumers due to its nice aroma, taste and colour which attract consumers' attention.

Figure 2.2 Herb and Fruit Infused Vinegar (Source: Stewart, 2020)

2.4 Edible Flowers

In general, flowers are commonly used for ornamental purpose to beautify the environment and also as a gift for loved ones. However, flowers are not only limited to these uses. Centuries ago, flowers had been used for decorative purpose of food, a source for natural aroma, natural food colourings and flavours. The flowers used in food preparation are known as edible flowers. The well-known edible flowers include nasturtium, chrysanthemum, daylily, calendula, osmanthus, rose and lavender.

Edible flowers have many benefits due to its nutritional value such as having higher amount of potassium than sodium that is good for the prevention of cardiovascular diseases (Fernandes *et al.,* 2017). Moreover, edible flowers are also rich in antioxidants (free radical scavenger) that helps to prevent the free radical from harming the body cells which helps in cancer prevention, anti-diabetes and also prevent Alzheimer's which is a degenerative disease due to aging (Kucekova *et al*., 2013; Gonzalez-Barrio *et al.*, 2018).

In the past few years, the demand for natural products have increased due to consumers' increased in knowledge about the side effect of non-natural food products and the environment factor such as the increased use of technology in daily life. Considering edible flower consist of natural antioxidants such as flavonoids, carotenoid, anthocyanin and phenols, it is an ideal ingredient to be used to improve the undesirable aroma and sour taste of vinegar, simultaneously, it also increases the nutritional values of vinegar and increase the variation of 100% natural products (Loizzo *et al*., 2015). The vinegar can be infused with edible flowers to create a new line of products that attract customers' attention and also increase their preferences in vinegar consumption. Furthermore, more choices of food, desserts and beverage recipes could be created using

this interesting combination of edible flowers and vinegar.

Furthermore, by infusing edible flowers into vinegar, new line of cosmetic products targeting acne and skin brightening effect could be produced. Since edible flower such as Rose and Osmanthus contains skin anti-inflammatory and skin brightening properties, and the acetic acid in vinegar has antimicrobial effect on acne, edible flower infused vinegar could be an addition of new line of personal care products in the cosmetic market (Tae *et al.*, 2018; Liu *et al.*, 2018; Mokhtar *et al*., 2020).

2.4.1 Rose

Roses are one of the most well-known flowers in the world. Roses are usually given to people as a token of love and friendship. It is also one of the most popular floral decorations. Rose is a perennial plant from genus *Rosa* and the family Rosaceae (Zandi *et al.*, 2015). Roses are usually use in the making of Rose oil which can be used for cosmetic purposes such as a Rose perfume and Rose soap. Rose oil is also used to flavour liquor and teas. There are many species of Rose but only some are used in food preparation. The Rose species that is commonly used in food preparation is Garden roses, Heirloom roses, French rose (*Rosa gallica*) and Damask rose (*Rosa damascena*).

In general, Rose has many benefits including anti-aging, anti-diabetic and anticancer (Vijayanchali, 2017). Damask rose (*Rosa damascena*) also has compound that is able to improve the function of cardiovascular. Moreoever, Damask rose (*Rosa damascena*) also contains high amount of compound such as flavonoids which has

antidepressant function. Damask rose (*Rosa damascena*) has high amount of antioxidant and considering many consumers demand for natural antioxidants from plants, it is a good ingredient to be infused into vinegar (Kart and Çağindi, 2017). Since fruit vinegar loses some of its antioxidants during fermentation, infusing dried Rose buds into the fruit vinegar will add antioxidants into the vinegar at the same time the aromatic Rose scent can also improve the undesired smell of the vinegar. This combination will not only boost the nutritional values of vinegar but also attract consumers' attention to try drinking vinegar which offers many health benefits.

Figure 2.3 Dried Rose Buds (*Rosa damascena*) (Source : Anubha, n.d.)

2.4.2 Osmanthus

Osmanthus fragrans is an ornamental plant which are widely cultivated in China and is often used as a traditional medicine to treat many diseases (Ouyang *et al.*, 2015). *Osmanthus fragrans* is a plant from the family Oleaceae which is also known as sweet olive, tea olive, and fragrant olive (Hung *et al*., 2013). In China, *Osmanthus fragrans* is called "*Kwai-fah"* which is commonly used to add flavour to tea and food including pastry, liqueurs, paste, cake and vinegar due to its delicate fruity and floral aroma (Hung *et al.*, 2013).

Moreover, several bioactive components are found in *O. fragrans* flowers, including flavonoids and phenolic acids have shown to exhibit neuroprotective, free radical scavenging, anti-aging, and antioxidant properties. Furthermore, according to Hung *et al.* (2013), in traditional Chinese medicine, *Osmanthus fragrans* is recommended to treat weakened vision, asthma, panting, cough, toothache, stomachache, diarrhea, hepatitis and halitosis.

Based on the research conducted by Lee *et al.* (2007), dried *Osmanthus fragrans* flower has a lot of phenols and flavonoids and it has high antioxidant activity. Osmanthus also contains high amount of carotenoid which contributes to the yellow pigment of the flower (Wang *et al.*, 2018). Since the smell of *Osmanthus fragrans* is sweet and pleasant and is usually used in food beverage in China, it is an ideal edible flower to be infused in Rambutan Vinegar to not only improve the aroma but also increase the nutritional and health benefits of Rambutan Vinegar.

Osmanthus fragrans is also rich in acteoside which helps in inhibiting the melanogenesis on skin and provide skin lightening properties (Liu *et al.*, 2018). Based on the research conducted by Xiong *et al.* (2015), *Osmanthus fragrans* is also a good source of natural antiaging compounds.

Figure 2.4 Dried Osmanthus Flower (*Osmanthus fragrans*) (Source : AudreyP, 2013)

2.5 Physicochemical Analysis

Physicochemical analysis is commonly used to investigate the correspondence between the composition, structure and properties of an object (Zlomanov, Khoviv and Zavrazhnov, 2013). In this research, the colour, pH, total soluble solid content and acetic acid content of infused vinegar will be measured.

2.5.1 Colour

Colours that is seen comes from the light carried wavelengths which are absorbed by the eyes that is then converted by our brain. Light fragmented into a colour spectrum of six different colours which include red, orange, yellow, green, blue, and violet. Red is known to have the longest wavelength while violet is known to have the shortest wavelength. For instance, when a yellow object is seen, this means except for yellow light, all the colours in the spectrum are absorbed. The yellow light which is unabsorbed is reflected from the object into the eyes and then converted by the brain to interpret it as the yellow colour that is seen by the eyes (Singh, 2006).

Based on a study conducted by Casas and Chinoperekweyi (2019), colour affects the buying behaviour of consumers. Colour psychology indicates the feelings, ideas, emotions and physiological behaviour that is affected by every colour (Wright, 2009). In term of marketing, colour has powerful persuasive force due to its function of attracting attention, sooth or irritates vision. With the right colours, it is a powerful marketing tool to attract consumers to buy the products and also increase consumers' confidence on the quality of the product. Therefore, in order to attract more consumers to buy and drink vinegar, the vinegar needs to have natural colours desired by consumers (Singh and Srivastava, 2011).

Colours could be measured by using a colorimeter. The concept of colorimetry is founded by Commission Internationale d'Eclairage (CIE) based on visual experiments. CIE L*a*b* (CIELAB) is a consistent colour model used traditionally to present all the colours which can be seen by the human eye (Radulescu-Grad *et al.,* 2013). By using colorimeter, colour is expressed in which *L** represents lightness, *a** defines the positive

values for red colour and negative value for green colour and *b** denotes the positive values for yellow colour and negative values for blue colour (Pathare, Opara and Al-Said, 2013).

2.5.2 pH

pH is a parameter to measure the acidity and alkalinity of an aqueous media (Karastogianni, Girousi and Sotiropoulos, 2016). The pH scale ranges from pH 1 to pH 14. pH 1 to pH 6 indicates the aqueous solution is acidic while pH 8 to pH 14 indicates the aqueous solution is alkaline. pH 7 indicates the aqueous solution is neutral with the balance of acidity and alkalinity. Therefore, when the aqueous solution falls under acidity, this means there is more hydrogen ions than hydroxide ions in the aqueous solution. Likewise, when the aqueous solution falls under alkalinity, this means there is more hydroxide ions than hydrogen ions in the aqueous solution (Boyd, Tucker and Viriyatum, 2011).

Besides, pH of an aqueous media can be determined by using universal indicator paper and also electronic pH meter. Universal indicator which is made from solutions of water, propan-1-ol, phenolphthalein sodium salt, sodium hydroxide, methyl red, bromothymol blue monosodium salt, and thymol blue monosodium salt to measure the pH according to colour changes (Sampim, Phupa and Sampim, 2018). Meanwhile, electronic pH meter measures pH by translating pH readings from the electronic meter which measures the glass electrodes which is able to attract electric potential (charge)

from the hydrogen ions activity in the aqueous solution (Karastogianni, Girous and Sotiropoulos, 2016). Hence, for an accurate pH measurement, pH meter was used to measure the pH of the infused vinegar.

2.5.3 Total Soluble Solid

Total Soluble Solid (TSS) is the measurement of soluble solids dissolve in water. Total Soluble Solid (TSS) is measured by using a digital refractometer in the unit of degrees Brix (°Bx) which is calculated using the dilution factor (Rongtong *et al.*, 2018). Total Soluble Solid (TSS) content of a solution is measured by the index of refraction which is the bending of the light beam in the refractometer. The measurement of Total Soluble Solid (TSS) is commonly used to determine the sugar content in fruits to check if the fruit is in good quality (unripe, ripe or overripen). 1°Brix is equivalent to 1% of total soluble solids in an aqueous solution. Therefore, the higher the value of degree Brix, the higher the total soluble solids, the sweeter the aqueous solution measured. Thus, the sugar content in the infused vinegar can be determined by using a refractometer and this helps to keep the sugar content as minimum as possible to prevent due fermentation of infused vinegar.

2.5.4 Acetic Acid Content

Acetic acid is also known as Glacial acetic acid, Ethylic acid, Pyroligneous acid, Vinegar acid and Methanecarboxylic acid. Acetic acid was also used as medicine and was most likely the first antibiotics that was discovered (Cheryan, 2009). The chemical formula of acetic acid is CH_3COOH. Acetic acid is the main composition in vinegar. Vinegar shall contain not less than 4% of v/w of acetic acid and shall not contain mineral acid (Food Act 1983 and Food Regulations 1985). Vinegar is produced by fermenting alcohol (aerobic fermentation) with bacteria, particularly, *Acetobacter* species which produce acetic acid (Pravasi, 2014).

$$C_2H_5OH + O_2 \longrightarrow CH_3COOH + H_2O \quad (2.1)$$

Besides, acetic acid content can be determined by using the titration method. Titration is a method to determine the unknown amount of chemical in a solution which is known as titrant by adding a fixed amount of chemical with a known concentration known as titrating solution (Pujari *et al*., 2020).

Furthermore, acetic acid content can also be measured by using the acetic acid digital refractometer. The acetic acid content is measured in gram of acetic acid in 100g of vinegar sample (g/100g) or the percentage of acetic acid in the vinegar sample.

2.6 Antioxidant Activity

Antioxidant is a substance which is able to slow down or prevent a substrate from oxidizing with a minimal concentration of antioxidants. Antioxidants prevent the damage of cells that is caused by free radicals (Young and Woodside, 2001). Free radicals are atoms or molecules which consists of unpaired electrons cause it to be highly reactive. This is because the unpaired electron is unstable resulting in electrons from other compounds in the cell to be abstracted in order for the free radical to obtain stability. The compound which loses its electron turns into free radical and become a chain reaction which damages the living cell during cellular metabolism (Phaniendra, Jestadi and Periyasamy, 2015). Free radical negatively affects the biological molecules in the body such as nucleic acids, proteins and lipids which results in increased oxidative stress. Oxidative stress induced by free radicals will cause diseases such as diabetes mellitus, cardiovascular diseases, neurodegenerative disorders (Alzheimer's disease) and also the development of cataract (Phaniendra, Jestadi and Periyasamy, 2015). Free radicals which are produced from oxygen are known as reactive oxygen species (ROS).

There are many types of antioxidants which are enzyme antioxidant (reductase), nutrient-derived antioxidant (ascorbic acid) and metal binding proteins (albumin) (Atta, Mohamed and Abdelgawad, 2017). Antioxidants inhibits oxidation of a substrate by neutralizing the free radicals. According to Atta, Mohamed and Abdelgawad (2017), there are two ways to inhibit oxidation which are chain-breaking and preventive. Chain-breaking antioxidant such as vitamin C, vitamin E and also β-carotene stabilize the free radicals and decays it into product that does not harm the cells. Meanwhile, preventive antioxidant scavenges initiating radicals to reduce the rate of oxidation chain initiation.

Antioxidants can be measured by using oxygen radical absorbance capacity (ORAC) assay by observing the prevention of peroxyl radical induced oxidation in order to measure the radical chain breaking ability of antioxidants. Besides, antioxidant activity can also be measured by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. DPPH is a stable chromogen radical which has the colour of deep purple. The principle of DPPH scavenging assay is DPPH radical will be reduced by the donation of an electron or a hydrogen atom from the antioxidants (Shahidi and Zhong, 2015). When the antioxidant activity is high, the purple colour of DPPH will turn colourless. The degree of discolouration indicates the scavenging potential.

2.7 Total Phenolic Content

Phenolic compounds are small molecules that are categorized based on their structures which have a minimum of one phenol unit. Different chemical structures of phenolic compounds are divided into different subgroups such as phenolic acids, flavonoids and quinones (Gan *et al*., 2019). Phenolic compound contents and antioxidant activity in food which are high in antioxidants have correlation. Moreover, phenolic compounds also exhibit antioxidant activity such as scavenging free radicals, donation of electrons or hydrogen atoms in order to prevent the damage of cells that free radicals caused (Minatel *et al.*, 2017).

Furthermore, total phenolic content in food products can be measured by using Folin-Ciocalteu method. Folin–Ciocalteu method measures the antioxidant activity by

observing the electron transfer and reducing ability of an antioxidant (Lamuela-Raventós, 2017). The concept of the Folin–Ciocalteu assay is phenolic compounds reduced the Folin–Ciocalteu reagent (FCR) which produces molybdenum–tungsten blue which can be measured using a spectrophotometer at 760 nm. The higher the concentration of the phenolic compounds in the reaction medium, the higher the intensity of the molybdenum–tungsten blue when measured at spectrophotometer 760 nm (Malta and Liu, 2014).

2.8 Total Flavonoid Content

Flavonoids are secondary metabolites of plants which consist of polyphenolic structure which is commonly found in food and beverages such as fruits and vinegar. Flavonoids also have antioxidants that helps in preventing atherosclerosis, cardiovascular disease and neurodegenerative disease such as Alzheimer's disease. Moreover, flavonoids are also widely used as an ingredient in supplement, medicine and cosmetic products. This is because flavonoids have high antioxidant activity, anti-inflammatory anti-carcinogenic and anti-mutagenic properties (Panche, Diwan, and Chandra, 2016).

Total flavonoid content can be measured using Aluminium Chloride ($AlCl_3$) colorimetric assay method. The concept of this method is $AlCl_3$ forms acid stable complexes with the C-4 keto groups and the C-3 or C-5 hydroxyl group of flavones and flavonols (Bhaigyabati, Devi and Bag, 2014). Furthermore, $AlCl_3$ also forms acid labile complexes with the ortho-dihydroxyl groups in the A- or B-ring of flavonoids (Bhaigyabati, Devi and Bag, 2014). Quercetin was used as the standard to plot the

calibration curve for this Aluminium Chloride ($AlCl_3$) colorimetric assay method (Rao, Abdurrazak and Mohd, 2016).

2.9 Anthocyanin Pigment

Anthocyanin is water-soluble pigments which have at least one phenol unit (Khoo *et al*., 2017). The colour pigment of fruits and vegetables are controlled by anthocyanins which exhibit red, purple, and blue colour (Khoo *et al.*, 2017). The word "Anthocyanin" originated from two Greek words which are *Anthos* (flowers) and *kyanos* (dark blue) which shows its importance as a natural colouring agent (Horbowicz *et al*., 2008). Anthocyanin is commonly found in plant tissue and shows a spectrum of visible colours which include orange and red up to purple and blue hues (Santos-Buelga, Mateus and De Freitas, 2014). Anthocyanins are categorized as flavonoids and their capacity to form flavylium cations distinguish them from other flavonoids (Mazza, 2007; Miguel, 2011).

Moreover, anthocyanin is water-soluble and less stable compared to carotenoids and is commonly extracted from fruits such as berries and flowers such as Rose (Castañeda-Ovando *et al*., 2009). Anthocyanins also has high antioxidant activity which have helps to prevent cardiovascular diseases, neurodegenerative diseases, cancer and diabetes mellitus (Konczak and Zhang, 2004; Castañeda-Ovando *et al.*, 2009). This is because the antioxidant properties in anthocyanin help to inhibit the free radicals from abstracting the electrons from other compounds in the cell resulting in the formation of more free radicals which gradually damages the cells.

Furthermore, colorimetric assay can also be used to determine the monomeric anthocyanin content. Anthocyanin pigment content can be determined by using a pH differential method which involves a two-buffer system. The two-buffer system is potassium chloride buffer at pH 1.0 (0.025 M) and sodium acetate buffer at pH 4.5 (0.4 M) (Lako *et al*., 2007; Rafi *et al.*, 2018). The monomeric anthocyanin pigments reversibly change the colour as the pH changes. The colored oxonium form occurs at pH 1.0 while the colourless hemiketal forms at pH 4.5 (Lee, Durst and Wrolstad, 2005). The difference in absorbance between pH 1.0 and 4.5 at 520nm is correlated to the anthocyanin concentration (Lee, Durst and Wrolstad, 2005). Polymeric anthocyanins could not be measured using the pH differential method because it is resistant to the color change with pH, hence, polymeric anthocyanins absorb light at pH 1.0 and pH 4.5. Since the midrange absorbance value for anthocyanins is observed and the weights are stated as cyanidin-3-glucoside equivalents because it is the standard anthocyanin pigment acquire in nature, the maximum absorbance chosen is 520 nm (Rousseau, 2014).

Chapter 3

METHODOLOGY

3.1 Materials, Chemicals, Equipment and Apparatus

3.1.1 Materials, Chemicals and Reagents

The materials which were used in this research were Rambutan Vinegar and dried edible flowers such as Rose and Osmanthus. The chemicals needed in this study are pH 4 and pH 7 standard solution from Horiba LAQUAtwin pH meter kit, distilled water, buffer solution (pH 1 and pH 4.5) produced from potassium chloride, sodium acetate and concentrated hydrochloric acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution, methanol, Folin-Ciocalteu reagent (FCR), gallic acid, sodium nitrate, aluminium chloride, sodium hydroxide, quercetin, ethanol and Rambutan Vinegar (95% water, 5% acetic acid).

3.1.2 Equipment and Apparatus

The equipment and apparatus which were used in this research were Horiba LAQUAtwin pH meter, digital Brix refractometer (g/100g), acetic acid digital refractometer, chroma meter, spectrophotometer, analytical balance, paper towel, cuvette, filter paper, funnel, sterilized pipettes, test tube with caps, test tube rack, micropipette, micropipette tips, measuring cylinder, Bunsen burner, wire gauze, 2 L Scott bottle, 250 mL Scott bottle, 100 mL volumetric flask, 10 mL volumetric flask, beaker (50 mL, 100 mL, 250 mL) and aluminium foil.

3.2 Preparation of Infused Vinegar

The Rose infused Rambutan Vinegars were prepared by measuring the dried Rose buds (1 g, 3 g, 5 g) by using an analytical balance. The measured dried Rose buds were inserted into three 250 mL empty Scott bottles. Then, 150 mL of Rambutan Vinegar (95% water, 5% acetic acid) was heated until it reached 70 °C and was poured into the empty glass bottles. The Scott bottle was then covered immediately. The vinegars were infused with dried Rose buds in a dark and cool area for 4 weeks. The Rose infused Rambutan Vinegars were collected each week from Week 0 to Week 4 to conduct physicochemical analysis and antioxidant test. The Osmanthus infused vinegars were prepared with the same procedure.

3.3 Physicochemical Analysis

Physicochemical analysis was conducted for the study of the colour, pH, total soluble solid (TSS) content and acetic acid content of dried edible flowers infused vinegar in comparison with the control (Rambutan Vinegar) under the influence of concentration of flower infused in Rambutan Vinegar and the time of infusion.

3.3.1 Colour

The colours of all samples of infused vinegar were measured using a chroma meter. The data of the colour shows in CIE L^* a^* b^* values in which L^* represent lightness of samples which range from black (L = 0) with no reflection and white (L = 100) with good reflection, a^* represent red (positive) and green (negative) and b^* represent yellow (positive) and blue (negative) (Magdić *et al.*, 2009). The Minolta chroma meter standard plate was used to calibrate the chromameter before measuring the samples (Magdić *et al.*, 2009). The 10 mL samples were placed in a transparent plastic and sealed. After that, the samples in the sealed transparent plastic were measured three times and the data were recorded in CIE $L^*a^*b^*$ values.

3.3.2 pH

The pH value of the samples (infused vinegar) and the control (Rambutan Vinegar) were measured by using Horiba LAQUAtwin pH meter. First, the Horiba LAQUAtwin pH meter was switched on. To calibrate the pH meter to obtain accurate result, the standard solution (pH 7 and pH 4) provided in the Horiba LAQUAtwin pH meter kit was used. A few drops of pH 7 standard solution were dropped into the flat sensor and the two circles on the flat sensor was ensured to be covered. Then, the CAL switch was pressed and the calibration was completed when the stability icon appears. The pH 7 standard solution on the sensor of the pH meter was removed, rinsed with distilled water and wiped gently with a paper towel. The same procedure was applied for the calibration of pH 4 standard solution. Next, a few drops of sample were dropped onto the sensor of the pH meter and the MEAS switch was pressed. Smiley face appeared and the backlight was lit on the screen when the measurement was completed. The samples were tested for 3 times using the pH meter and all the readings of the samples were recorded. The sample on the sensor was removed. Then, the sensor of the pH meter was cleaned with tap water and wiped gently with a paper towel. All the samples were measured three times to obtain accurate results.

3.3.3 Total Soluble Solid

The total soluble solid of all the samples (infused vinegar) and control (Rambutan Vinegar) were measured by using a digital Brix refractometer (g/100g). The Brix refractometer was first calibrated by putting a few drops of distilled water until the surface of the sensor was covered and then the zero button was pressed. After that, the surface of the sensor was cleaned gently with a paper towel. Then, a few drops of samples were placed onto the surface of the sensor until the whole sensor was covered and the start button was pressed. The readings were recorded. All samples were measured three times with the digital brix refractometer in order to obtain accurate results.

3.3.4 Acetic Acid Content

Acetic acid content in the samples (infused vinegar) and control (Rambutan Vinegar) were measured by using a digital acetic acid refractometer. The digital acetic acid refractometer was first calibrated by putting a few drops of distilled water until the whole surface of the sensor was covered and then the zero button was pressed. After that, a few drops of sample were placed on the surface of the sensor until the whole sensor was covered and then the start button was pressed. All the samples were measured three times to ensure accuracy in results.

3.4 Antioxidant Activity

The antioxidant activity of the samples (infused vinegar) and control (Rambutan Vinegar) to scavenge DPPH radicals was determined by using Turkmen *et al.* (2006)'s method with minor modifications. The method used by Turkmen *et al*. (2006), involve the use of 2,2-diphenyl-1-picryl-hydrazyl (DPPH) free radical assay. Firstly, 0.1 mM of DPPH solution in methanol was prepared and then, 1ml of sample was mixed with 3ml of DPPH solution. After that, the solution was stored at room temperature for 30 minutes in a dark area. The absorbance value of the mixture was measured by using a spectrophotometer at 517 nm against a blank, 2,2-diphenyl-1-picryl-hydrazyl (DPPH). All samples were done in triplicates and recorded. Then, the antioxidant activity (AA) was reported as percentage inhibition of the DPPH radical and was determined by the following equation :

$$\text{Scavenging activity (\%)} = \frac{Absorbance\ of\ Control - Absorbance\ of\ sample}{Absorbance\ of\ control} x\ 100\% \quad (3.1)$$

When the absorbance value is low which means light can pass through and therefore, the sample that has less particles and is less opaque. When the antioxidant activity is high, the purple colour of DPPH solution changes into colourless. Hence, the lower the absorbance value, the stronger antioxidant activity.

3.5 Total Phenolic Content

The total phenolic content of the samples (infused vinegar) and the control (Rambutan Vinegar) were determined by using the Folin-Ciocalteua's method with slight alteration (Hafzan, Saw and Fadzilah, 2017). First, the samples were diluted with distilled water at a ratio of 1:9. Then, 0.3 mL of each diluted sample were mixed with 1 mL of 10% Folin-Ciocalteu reagent in a test tube. Then, the mixture was kept at 25 °C for 5 minutes and then 1.2 mL of 7.5% sodium carbonate solution was added to the mixture. Since, the Folin-Ciocalteu reagent is light-sensitive, mixture was kept at room temperature for 2 hours in a dark area. The absorbance value of the samples was measured at λmax 765 nm by using a spectrophotometer against a blank (distilled water). All the samples were done in triplicates and recorded. Similar steps were repeated for standard solution of gallic acid and the calibration line was constructed by using a standard curve range from 0 µg/mL to 250 µg/mL of gallic acid. The results were expressed as mg of gallic acid per mL of sample (GAE µg/mL).

3.6 Total Flavonoid Content

The total flavonoid content of samples (infused vinegar) and control (Rambutan Vinegar) were determined by aluminium chloride ($AlCl_3$) colorimetric method as described by (Yikmis, 2019). First, 1 mL of sample was mixed with 4 mL of distilled water in a test tube. Then, 0.3 mL of 5% sodium nitrate ($NaNO_3$) was added immediately into the mixture and kept for 5 min at room temperature. 0.3 mL of 10% aluminium chloride ($AlCl_3$) was added and the mixture was kept for another 6 minutes. After that, 2 mL of 1 M sodium hydroxide (NaOH) was added into the mixture. The mixture was then diluted to 10 mL with distilled water and was kept for 30 min at room temperature. The absorbance was measured at λmax 510 nm by using a spectrophotometer against a blank (distilled water). All samples were done in triplicates to ensure accuracy in results. Similar procedure was repeated for the standard solution of quercetin and the calibration line was constructed using standard curve range from 0.5 mg/mL to 5 mg/mL of quercetin. Total flavonoid content was stated as mg of quercetin / mL of sample (QE mg/mL).

3.7 Anthocyanin Pigment

Total anthocyanin pigment content of the samples (infused vinegar with the highest antioxidant activity) and control (Rambutan Vinegar) were determined by using the AOAC pH differential method (Lee *et al.,* 2005). Firstly, buffer solution of pH 1.0 and pH 4.5 were prepared by using potassium chloride (KCl) and sodium acetate (CH_3CO_2Na).

100 mL of buffer solution of pH 1.0 (0.025 M potassium chloride) was prepared by weighing 0.18 g of potassium chloride (KCl) and then the weighted KCl was placed in a 100 mL volumetric flask. After that, distilled water was added until the water level reached the line on the volumetric flask. Then, the solution was transferred to a beaker and the pH of the solution was adjusted to pH 1.0 (±0.05) with hydrochloric acid, HCl.

100 mL of buffer solution of pH 4.5 (0.4M sodium acetate) was prepared by weighing 3.28 g of sodium acetate (CH_3CO_2Na) and then the weighted CH_3CO_2Na was placed in 100 mL of volumetric flask. After that, distilled water was added until the water level reached the line on the volumetric flask. Then, the solution was transferred to a beaker and the pH of the solution was adjusted to pH 4.5 (±0.05) with hydrochloric acid, HCl.

1 mL of each sample was diluted separately with 9 mL of 0.025 M potassium chloride, KCl (pH 1.0) and 9 mL of 0.4 M sodium acetate, CH_3CO_2Na (pH 4.5) buffer in a 10 mL volumetric flask. The mixture was left for 15 minutes at room temperature. The absorbance was measured by using vis-spectrophotometer at λmax 520 nm and λmax 700 nm. The anthocyanin pigment concentration of sample was calculated by following

formula (Lee *et al.*, 2005):

$$\text{Anthocyanin pigment concentration (cyaniding-3-glucoside equivalent, mg/L)} = \frac{A \times MW \times DF \times 10^3}{\varepsilon \times 1} \quad (3.2)$$

where A = [(A λmax =520 nm – A λmax=700 nm) pH 1.0 – (A λmax=520 nm – A λmax=700 nm) pH 4.5],

MW represented molecular weight of cyaniding-3-glucoside = 449.2 g/mol,

DF represented dilution factor = 10,

1 is path length in cm,

ε represented molar extinction coefficient, 26,900 $L \cdot mol^{-1} \cdot cm^{-1}$

10^3 is the factor for conversion of g to mg.

3.8 Statistical Analysis

The triplicated data from the experiments were expressed as mean ± standard deviation in a table form. Two-way Analysis of Variance (ANOVA) and One-way Analysis of Variance (ANOVA) were used to analyze the data collected. The data were analyzed by using significant difference test at $p<0.05$ in Two-way Analysis of Variance (ANOVA) and One-way Analysis of Variance (ANOVA) The IBM SPSS Statistics 26 Software was used to conduct statistical analysis. Tukey's test was used as the Post Hoc test when the results analyzed from Analysis of Variance (ANOVA) test show significant difference among the sample means. Pearson's Correlation Coefficient was conducted to test the correlation between antioxidant activity and time of infusion of flower infused vinegar. Regression was then conducted when the correlation of antioxidant activity and time of infusion of flower infused vinegar shows significance difference.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Colour

4.1.1 Lightness (*L**)

Based on Table 4.1, there were significant ($p<0.05$) difference in the overall lightness (L^*) of Rose infused Rambutan Vinegar (RVR) under the influence of the concentration of Rose (grams) infused in Rambutan Vinegar (RV) and the time of infusion (weeks). The L^* of all RVR were significantly different at $p<0.05$ with RV. The L^* of RV(3 g Rose) and RV(5 g Rose) decreases significantly when compared to RV while L^* of RV(1 g Rose) decreases significantly at Week 0 (60.31), increases significantly at Week 1 (72.09) and Week 2 (71.73), and eventually decreases significantly at Week 3 (54.37) and Week 4 (54.22) when compared to RV (66.00). The value of lightness decreases as the concentration of Rose increases because Rose consists of red, blue, purple, yellow and orange pigments because of the presence of anthocyanin

and carotenoids (Lachman *et al.*, 2001). In edible roses, the principal anthocyanins are cyanidin and pelargonidin, which are also found in strawberries. Since the amount of pelargonidin is only 10% of cyanidin, cyanidin is the most important component contributing to antioxidant activity in Rose (Yang and Shin, 2017). The vivid red hue of the petals was indicated by an increase in pelargonidin 3,5-diglucoside content, which resulted in an increase in a value *a** and a decrease in *L** value (Wan e*t al.*, 2019).

The *L** of all RVR were significantly different at $p<0.05$ starting from Week 1 to Week 4 in comparison with Week 0. The *L** of RV(3 g Rose) decreases significantly from Week 1 (62.66) to Week 4 (53.11) in comparison with Week 0 (62.79) whereas the *L** of RV(1 g Rose) and RV(5 g Rose) initially increases from Week 1 to Week 2 and decreases from starting from Week 3 to Week 4 in comparison with Week 0.

Table 4.1: Lightness of Rose infused Rambutan Vinegar under different formulations.

Week	Lightness			
	RV	RV(1 g Rose)	RV(3 g Rose)	RV(5 g Rose)
0	66.00 ± 0.31^{cf}	60.31 ± 0.89^{bf}	62.79 ± 0.74^{af}	52.73 ± 0.87^{af}
1	70.05 ± 2.27^{cg}	72.09 ± 0.58^{bg}	62.66 ± 1.64^{ag}	64.55 ± 0.76^{ag}
2	67.87 ± 0.47^{ce}	71.73 ± 1.34^{be}	42.16 ± 0.41^{ae}	52.73 ± 1.11^{ae}
3	63.26 ± 0.59^{cd}	54.37 ± 2.38^{bd}	57.62 ± 1.35^{ad}	49.62 ± 0.52^{ad}
4	64.56 ± 0.57^{cd}	54.22 ± 1.91^{bd}	53.11 ± 1.65^{ad}	52.70 ± 1.48^{ad}

Values are expressed as mean ± SD of triplicates measurements. RV=Rambutan Vinegar, abc = different superscripts signify significant mean differences at $p<0.05$ between concentration. defg = different superscripts signify significant mean differences at $p<0.05$ between time.

Based on Table 4.2, there were significant ($p<0.05$) difference in the overall lightness (L^*) of Osmanthus infused Rambutan Vinegar (RVO) under the influence of the concentration of Osmanthus (grams) infused in Rambutan Vinegar (RV) and the time of infusion (weeks). The L^* of all RVO were significantly different at $p<0.05$ with RV. The L^* of RV(1 g Osmanthus) decreases significantly when compared to RV while L^* of RV(3 g Osmanthus) increases significantly and RV(5 g Osmanthus) decreases significantly at Week 0 (62.77) and increases significantly starting from Week 1 (75.88) to Week 4 (67.10) when compared to RV (66.00). The L^* of RV(3 g Osmanthus) increases significantly because fermentation take place. Fermentation increase the lightness and redness of a product (Barbut, 2010). The value of lightness decreases as the concentration of Osmanthus increases because Osmanthus consists of yellow pigments due to the presence of carotenoids which are yellow, orange and red pigments (Wang *et al*., 2018).

The L^* of all RVO were significantly different at $p<0.05$ starting from Week 1 to Week 4 in comparison with Week 0. The L^* of RV(5 g Osmanthus) increases significantly from Week 1 (75.88) to Week 4 (67.10) in comparison with Week 0 (62.77) whereas the L^* of RV(1 g Osmanthus) and RV(3 g Osmanthus) initially increases from Week 1 to Week 2 and decreases starting from Week 3 to Week 4 in comparison with Week 0. The longer the time of infusion the lower the value of L^* (Laddi, 2011).

Table 4.2: Lightness of Osmanthus infused Rambutan Vinegar under different formulations.

Week	Lightness			
	RV	RV (1 g Osmanthus)	RV (3 g Osmanthus)	RV (5 g Osmanthus)
0	66.00 ± 0.31^{bf}	65.25 ± 1.26^{af}	70.76 ± 1.25^{df}	62.77 ± 1.32^{cf}
1	70.05 ± 2.27^{bh}	67.41 ± 2.02^{ah}	82.42 ± 0.42^{dh}	75.88 ± 1.63^{ch}
2	67.87 ± 0.47^{bg}	65.51 ± 2.00^{ag}	74.18 ± 1.24^{dg}	78.31 ± 0.51^{cg}
3	63.26 ± 0.59^{bef}	61.79 ± 0.78^{aef}	68.85 ± 1.70^{def}	67.86 ± 1.89^{cef}
4	64.56 ± 0.57^{be}	60.06 ± 0.83^{ae}	65.00 ± 2.11^{de}	67.10 ± 0.89^{ce}

Values are expressed as mean ± SD of triplicates measurements. RV=Rambutan Vinegar, abcd = different superscripts signify significant mean differences at $p<0.05$ between concentration. efgh = different superscripts signify significant mean differences at $p<0.05$ between time.

4.1.2 Redness (*a**)

Based on Table 4.3, there were significant ($p<0.05$) difference in the overall redness (*a**) of Rose infused Rambutan Vinegar (RVR) under the influence of the concentration of Rose (grams) infused in Rambutan Vinegar (RV) and the time of infusion (weeks). The *a** of all RVR were significantly different at $p<0.05$ with RV. The *a** of RV(1 g Rose), RV(3 g Rose) and RV(5 g Rose) increases significantly when compared to RV.

The *a** of all RVR were significantly different at $p<0.05$ starting from Week 1 to Week 4. The *a** of RV(3 g Rose) and RV(5 g Rose) increases significantly from Week 1 to Week 4 in comparison with Week 0 whereas the *a** of RV(1 g Rose) initially decreases significantly at Week 1 (1.35) and Week 2 (1.47) and increases significantly from Week 3 (5.84) to Week 4 (15.01) in comparison with Week 0 (2.77). The *a** of RV(1 g Rose) at Week 4 (15.01) and RV(5 g Rose) at Week 4 (11.57) is high because the RVR undergo fermentation. According to Barbut (2010), lightness and redness of a product increases during fermentation.

Table 4.3: Redness of Rose infused Rambutan Vinegar under different formulations.

Week	Redness			
	RV	RV (1 g Rose)	RV (3 g Rose)	RV (5 g Rose)
0	2.05 ± 0.02^{dh}	2.77 ± 0.05^{bh}	2.99 ± 0.03^{ch}	3.29 ± 0.08^{ah}
1	0.28 ± 0.05^{di}	1.35 ± 0.06^{bi}	3.45 ± 0.13^{ci}	4.57 ± 0.23^{ai}
2	0.60 ± 0.04^{dg}	1.47 ± 0.17^{bg}	6.38 ± 0.14^{cg}	7.30 ± 0.08^{ag}
3	0.51 ± 0.05^{df}	5.84 ± 0.20^{bf}	4.84 ± 0.21^{cf}	8.94 ± 0.17^{af}
4	0.77 ± 0.01^{de}	15.01 ± 0.20^{be}	6.13 ± 0.12^{ce}	11.57 ± 0.27^{ae}

Values are expressed as mean ± SD of triplicates measurements. RV=Rambutan Vinegar, abcd = different superscripts signify significant mean differences at $p<0.05$ between concentration. efghi = different superscripts signify significant mean differences at $p<0.05$ between time.

Based on Table 4.4, there were significant ($p<0.05$) difference in the overall redness (*a**) of Osmanthus infused Rambutan Vinegar (RVO) under the influence of the concentration of Osmanthus (grams) infused in Rambutan Vinegar (RV) and the time of infusion (weeks). The *a** of all RVO were significantly different at $p<0.05$ with RV. The *a** of RV(1 g Osmanthus), RV(3 g Osmanthus) and RV(5 g Osmanthus) decrease significantly when compared to RV. The redness of RVO decrease significantly because Osmanthus contains more yellow pigment in which yellow pigment is from the carotenoids present in Osmanthus (Wang *et al*., 2018). Carotenoids are pigments in the range from yellow to red (Wang *et al.,* 2018). Based on Table 4.4, RVO reflect green colour because all the RVO samples from Week 0 to Week 4 except RV(1 g Osmanthus) at Week 4 (0.05) shows negative values which indicates greenness.

The *a** of all RVO were significantly different at $p<0.05$ starting from Week 1 to

Week 4 in comparison with Week 0. The *a** of RV(1 g Osmanthus) and RV(3 g Osmanthus) decreases significantly at Week 1 and increase significantly starting from Week 2 to Week 4 in comparison with Week 0 whereas the *a** of RV(5 g Osmanthus) initially decreases from Week 1 (-3.51) to Week 3 (-1.94) and increases at Week 4 (-1.42) in comparison with Week 0 (-1.60). RV shows significant decrease in redness from Week 1 to Week 4 in comparison with Week 0.

Table 4.4: Redness of Osmanthus infused Rambutan Vinegar under different formulations.

Week	Redness			
	RV	RV (1 g Osmanthus)	RV (3 g Osmanthus)	RV (5 g Osmanthus)
0	2.05 ± 0.02^{dg}	-0.64 ± 0.02^{cg}	-2.50 ± 0.08^{bg}	-1.60 ± 0.12^{ag}
1	0.28 ± 0.05^{de}	-0.68 ± 0.07^{ce}	-3.13 ± 0.03^{be}	-3.51 ± 0.08^{ae}
2	0.60 ± 0.04^{df}	-0.20 ± 0.05^{cf}	-2.38 ± 0.03^{bf}	-3.01 ± 0.02^{af}
3	0.51 ± 0.05^{dg}	-0.18 ± 0.05^{cg}	-1.34 ± 0.05^{bg}	-1.94 ± 0.06^{ag}
4	0.77 ± 0.01^{dh}	0.05 ± 0.02^{ch}	-0.58 ± 0.16^{bh}	-1.42 ± 0.09^{ah}

Values are expressed as mean ± SD of triplicates measurements. RV=Rambutan Vinegar, abcd = different superscripts signify significant mean differences at $p<0.05$ between concentration. efgh = different superscripts signify significant mean differences at $p<0.05$ between time.

4.1.3 Yellowness (*b**)

Based on Table 4.5, there were significant ($p<0.05$) difference in the overall yellowness (*b**) of Rose infused Rambutan Vinegar (RVR) under the influence of the concentration of Rose (grams) infused in Rambutan Vinegar (RV) and the time of infusion (weeks). The *b** of all RVR were significantly different at $p<0.05$ with RV. The *b** of RV(1 g Rose), RV(3 g Rose) and RV(5 g Rose) increases significantly when compared to RV.

The *b** of all RVR were significantly different at $p<0.05$ starting from Week 1 to Week 4 in comparison with Week 0. The *b** of RV(1 g Rose), RV(3 g Rose) and RV(5 g Rose) increases significantly from Week 1 to Week 4 in comparison with Week 0.

Table 4.5: Yellowness of Rose infused Rambutan Vinegar under different formulations.

Week	Yellowness			
	RV	RV (1 g Rose)	RV (3 g Rose)	RV (5 g Rose)
0	0.90 ± 0.02^{ah}	3.48 ± 0.07^{ch}	5.64 ± 0.04^{bh}	9.59 ± 0.29^{dh}
1	7.96 ± 0.19^{ag}	13.53 ± 0.38^{cg}	19.03 ± 0.09^{bg}	23.72 ± 0.18^{dg}
2	6.65 ± 0.01^{ag}	20.00 ± 0.37^{cg}	14.96 ± 0.15^{bg}	22.13 ± 0.47^{dg}
3	8.28 ± 0.03^{af}	20.80 ± 0.78^{cf}	19.68 ± 0.24^{bf}	21.86 ± 0.30^{df}
4	8.04 ± 0.04^{ae}	23.31 ± 0.74^{ce}	19.78 ± 0.43^{be}	24.51 ± 0.63^{de}

Values are expressed as mean ± SD of triplicates measurements. RV=Rambutan Vinegar, abcd = different superscripts signify significant mean differences at $p<0.05$ between concentration. efgh = different superscripts signify significant mean differences at $p<0.05$ between time.

Based on Table 4.6, there were significant ($p<0.05$) difference in the overall yellowness (*b**) of Osmanthus infused Rambutan Vinegar (RVO) under the influence of the concentration of Osmanthus (grams) infused in Rambutan Vinegar (RV) and the time of infusion (weeks). The *b** of all RVO were significantly different at $p<0.05$ with RV. The *b** of RV(1 g Osmanthus), RV(3 g Osmanthus) and RV(5 g Osmanthus) increase significantly when compared to RV. The yellowness of RVO increase significantly because Osmanthus contains carotenoids which are yellow, orange and red pigments (Wang *et al.,* 2018). Osmanthus contains carotenoid because it has high amount of flavonoids in which carotenoids are part of flavonoids.

The *b** of all RVO were significantly different at $p<0.05$ starting from Week 1 to Week 4 in comparison with Week 0. The *b** of RV(1 g Osmanthus) and RV(5 g Osmanthus) increases significantly at Week 1 and decrease significantly starting from Week 2 to Week 4 in comparison with Week 0 whereas the *b** of RV(3 g Osmanthus) decreases significantly from Week 1 (18.32) to Week 4 (15.94) when compared to Week 0 (19.20). Since, Osmanthus has high amounts of carotenoid which contributes to the yellow, orange and red pigments in flowers, the yellowness of the RVO increases because carotenoid which is a fat-soluble pigment is soluble in Rambutan Vinegar which contains about 0.07% fats (Wang *et al.*, 2018; Mezzomo & Ferreira, 2016; Mokhtar *et al.*, 2016).

Table 4.6: Yellowness of Osmanthus infused Rambutan Vinegar under different formulations.

Week	Yellowness			
	RV	RV (1 g Osmanthus)	RV (3 g Osmanthus)	RV (5 g Osmanthus)
0	0.90 ± 0.02^{dg}	13.08 ± 0.10^{cg}	19.20 ± 0.10^{bg}	21.66 ± 0.28^{ag}
1	7.96 ± 0.19^{de}	13.10 ± 0.20^{ce}	18.32 ± 0.27^{be}	22.20 ± 0.26^{ae}
2	6.65 ± 0.01^{df}	12.32 ± 0.30^{cf}	18.06 ± 0.04^{bf}	20.43 ± 0.34^{af}
3	8.28 ± 0.03^{df}	12.88 ± 0.13^{cf}	16.72 ± 0.08^{bf}	20.29 ± 0.27^{af}
4	8.04 ± 0.04^{dg}	12.25 ± 0.04^{cg}	15.94 ± 0.15^{bg}	18.90 ± 0.09^{ag}

Values are expressed as mean ± SD of triplicates measurements. RV=Rambutan Vinegar, abcd = different superscripts signify significant mean differences at $p<0.05$ between concentration. efg = different superscripts signify significant mean differences at $p<0.05$ between time.

4.2 pH

Based on Table 4.7, there were significant ($p<0.05$) difference in the overall pH of Rose infused Rambutan Vinegar (RVR) under the influence of the concentration of Rose (grams) infused in Rambutan Vinegar (RV) and the time of infusion (weeks). The pH of all RVR were significantly different at $p<0.05$ with RV. The pH of RV(1 g Rose), RV(3 g Rose) and RV(5 g Rose) increases significantly when compared to RV.

The pH of all RVR were significantly different at $p<0.05$ starting from Week 1 to Week 4 in comparison with Week 0. The pH of RV(1 g Rose) is constant from Week 1 to Week 2 (pH 3.50) and increases significantly from Week 3 (pH 4.20) to Week 4 (pH

5.33) when compared to Week 0 (pH 3.50). The pH of RV(3 g Rose) increases significantly from Week 1 (pH 3.57) to Week 4 (pH 3.67) when compared to Week 0 (pH 3.50). The pH of RV(5 g Rose) is constant at Week 1 (pH 3.60) and increases significantly from Week 2 (pH 3.70) to Week 4 (pH 4.60) in comparison with Week 0 (pH 3.60). When the concentration of rose infused in RV increases, the pH value of RVR increases. Similarly, when the time of infusion of Rose in RV increases, the pH value of RVR increases. According to Ousaaid *et al.* (2022), the pH of fruit vinegar generally fall into the range of pH 2.40 to pH 3.90. The pH of RV(1 g Rose) at Week 3 (pH 4.20) and at Week 4 (pH 5.33), and the pH of RV(5 g Rose) at Week 3 (pH 4.00) and pH of RV(5 g Rose) at Week 4 (pH 4.60) are not in the pH range of fruit vinegar because they undergone acetic acid fermentation in which the optimal growth conditions for the fermentation to take place is from pH 3 to pH 5 (Drysdale & Fleet, 1988).

Table 4.7: pH of Rose infused Rambutan Vinegar under different formulations.

Week	pH			
	RV	RV (1 g Rose)	RV (3 g Rose)	RV (5 g Rose)
0	3.43 ± 0.06^{ae}	3.50 ± 0.00^{de}	3.50 ± 0.00^{be}	3.60 ± 0.00^{ce}
1	3.40 ± 0.00^{aef}	3.50 ± 0.00^{def}	3.57 ± 0.06^{bef}	3.60 ± 0.00^{cef}
2	3.40 ± 0.00^{af}	3.50 ± 0.00^{df}	3.60 ± 0.00^{bf}	3.70 ± 0.00^{cf}
3	3.40 ± 0.00^{ag}	4.20 ± 0.00^{dg}	3.63 ± 0.06^{bg}	4.00 ± 0.00^{cg}
4	3.43 ± 0.06^{ah}	5.33 ± 0.06^{dh}	3.67 ± 0.06^{bh}	4.60 ± 0.00^{ch}

Values are expressed as mean ± SD of triplicates measurements. RV=Rambutan Vinegar, abcd = different superscripts signify significant mean differences at $p<0.05$ between concentration. efgh = different superscripts signify significant mean differences at $p<0.05$ between time.

Based on Table 4.8, there were significant ($p<0.05$) difference in the overall pH of Osmanthus infused Rambutan Vinegar (RVO) under the influence of the concentration of Osmanthus (grams) infused in Rambutan Vinegar (RV) and the time of infusion (weeks). The pH of all RVO were significantly different at $p<0.05$ with RV. The pH of RV(3 g Osmanthus) and RV(5 g Osmanthus) increase significantly when compared to RV whereas the pH of RV(1 g Osmanthus) increases significantly from Week 0 (pH 3.50) to Week 3 (pH 3.50) and decreases at Week 4 (pH 3.07) when compared to RV (pH 3.50).

The pH of all RVO were significantly different at $p<0.05$ starting from Week 1 to Week 4 in comparison with Week 0. The pH of RV(1 g Osmanthus) remain constant from Week 1 (pH 3.50) to Week 3 (pH 3.50) and decrease significantly at Week 4 (pH 3.07) when compared to Week 0 (pH 3.50). The pH of RV(3 g Osmanthus) increase significantly from Week 1 (pH 3.53) to Week 4 (pH 3.67) when compared to Week 0 (pH 3.50). The pH of RV(5 g Osmanthus) increases significantly at Week 2 (pH 3.70) and at Week 4 (pH 3.70) in comparison with Week 0 (pH 3.60). The overall pH of RVO increases, but this does not change to acidic properties of the RV because the pH of RVO still fall within the range of fruit vinegar which is pH 2.40 to pH 3.90 (Ousaaid *et al.*, 2022).

Table 4.8: pH of Osmanthus infused Rambutan Vinegar under different formulations.

Week	pH			
	RV	RV (1 g Osmanthus)	RV (3 g Osmanthus)	RV (5 g Osmanthus)
0	3.43 ± 0.06^{bg}	3.50 ± 0.00^{ag}	3.50 ± 0.00^{cg}	3.60 ± 0.00^{dg}
1	3.40 ± 0.00^{bef}	3.50 ± 0.00^{aef}	3.53 ± 0.06^{cef}	3.63 ± 0.06^{def}
2	3.40 ± 0.00^{bef}	3.50 ± 0.00^{aef}	3.60 ± 0.00^{cef}	3.70 ± 0.00^{def}
3	3.40 ± 0.00^{be}	3.50 ± 0.00^{ae}	3.60 ± 0.00^{ce}	3.63 ± 0.06^{de}
4	3.43 ± 0.06^{bfg}	3.07 ± 0.06^{afg}	3.67 ± 0.12^{cfg}	3.70 ± 0.10^{dfg}

Values are expressed as mean ± SD of triplicates measurements. RV=Rambutan Vinegar, abcd = different superscripts signify significant mean differences at $p<0.05$ between concentration. efg = different superscripts signify significant mean differences at $p<0.05$ between time.

4.3 Total Soluble Solid

Based on Table 4.9, there were significant ($p<0.05$) difference in the overall total soluble solid (TSS) content of Rose infused Rambutan Vinegar (RVR) under the influence of the concentration of Rose (grams) infused in Rambutan Vinegar (RV) and the time of infusion (weeks). The TSS content of all RVR were significantly different at $p<0.05$ with RV. The TSS content of RV(1 g Rose) and RV(5 g Rose) increases significantly from Week 0 to Week 1 and decreases significantly starting from Week 2 to Week 4 when compared to RV. The TSS content of RV(3 g Rose) increases significantly from Week 0 (3.30 °Bx) to Week 4 (3.90 °Bx) when compared to RV (2.93 °Bx).

The TSS content of all RVR were significantly different at $p<0.05$ starting from Week 1 to Week 4 in comparison with Week 0. The TSS content of RV(1 g Rose) and RV(5 g Rose) increases significantly at Week 1 and decreases significantly from Week 2 to Week 4 in comparison with Week 0. The TSS content decreases significantly because the RVR undergo fermentation which cause the decrease in TSS content. According to Wall *et al*. (2015), the TSS content decreases during fermentation because sugar is metabolized by acetic acid bacteria. The TSS content of RV(3 g Rose) increases significantly from Week 1 (3.77 °Bx) to Week 4 (3.90 °Bx) in comparison with Week 0 (3.30 °Bx).

Table 4.9: Total Soluble Solid content of Rose infused Rambutan Vinegar under different formulations.

Week	Total Soluble Solid (°Bx)			
	RV	RV (1 g Rose)	RV (3 g Rose)	RV (5 g Rose)
0	2.93 ± 0.12^{be}	3.07 ± 0.06^{ce}	3.30 ± 0.00^{ae}	3.40 ± 0.10^{be}
1	2.90 ± 0.00^{bd}	3.20 ± 0.00^{cd}	3.77 ± 0.12^{ad}	3.80 ± 0.00^{bd}
2	2.83 ± 0.06^{be}	2.50 ± 0.00^{ce}	3.87 ± 0.06^{ae}	3.30 ± 0.00^{be}
3	2.80 ± 0.00^{bf}	1.43 ± 0.06^{cf}	3.90 ± 0.00^{af}	2.30 ± 0.00^{bf}
4	2.93 ± 0.06^{bg}	1.00 ± 0.00^{cg}	3.90 ± 0.00^{ag}	1.70 ± 0.00^{bg}

Values are expressed as mean ± SD of triplicates measurements. RV=Rambutan Vinegar, abc = different superscripts signify significant mean differences at $p<0.05$ between concentration. defg = different superscripts signify significant mean differences at $p<0.05$ between time.

Based on Table 4.10, there were significant ($p<0.05$) difference in the overall total soluble solid (TSS) content of Osmanthus infused Rambutan Vinegar (RVO) under the influence of the concentration of Osmanthus (grams) infused in Rambutan Vinegar (RV) and the time of infusion (weeks). The TSS content of all RVO were significantly different at $p<0.05$ with RV. The TSS content of RV(1 g Osmanthus) and RV(3 g Osmanthus) initially increases significantly and decreases at Week 4 when compared to RV. The TSS content of RV(5 g Osmanthus) increase significantly from Week 0 (4.43 °Bx) to Week 4 (3.63 °Bx) when compared to RV (2.93 °Bx).

The TSS content of all RVO were significantly different at $p<0.05$ starting from Week 1 to Week 4 in comparison with Week 0. The TSS content of RV(1 g Osmanthus), RV(3 g Osmanthus) and RV(5 g Osmanthus) significantly decrease from Week 1 to Week 4 when compared to Week 0. Based on the study conducted by Muhialdin *et al.* (2019), the TSS content is related to fermentation because during aerobic fermentation. This is because with the presence of *Saccharomyces cerevisiae*, glucose is converted into ethanol which is later converted into acetic acid by *Acetobacter aceti* (Patel and Pandya, 2015). Hence, as the infusion time of flower in RV increases, fermentation eventually occurred and caused the TSS content to decrease because glucose was converted into ethanol. When the concentration of Osmanthus infused in RV increases, the TSS content of the RVO increases too. On the contrary, when the time of infusion of RVO increases, the TSS content of RVO decreases.

Table 4.10: Total Soluble Solid content of Osmanthus infused Rambutan Vinegar under different formulations.

Week	Total Soluble Solid (°Bx)			
	RV	RV (1 g Osmanthus)	RV (3 g Osmanthus)	RV (5 g Osmanthus)
0	2.93 ± 0.12^{de}	3.27 ± 0.06^{ce}	3.87 ± 0.06^{be}	4.43 ± 0.06^{ae}
1	2.90 ± 0.00^{df}	3.07 ± 0.12^{cf}	3.70 ± 0.00^{bf}	4.30 ± 0.00^{af}
2	2.83 ± 0.06^{dg}	3.03 ± 0.15^{cg}	3.43 ± 0.06^{bg}	4.10 ± 0.00^{ag}
3	2.80 ± 0.00^{dh}	2.90 ± 0.00^{ch}	3.17 ± 0.06^{bh}	3.90 ± 0.00^{ah}
4	2.93 ± 0.06^{di}	2.83 ± 0.15^{ci}	2.90 ± 0.00^{bi}	3.63 ± 0.06^{ai}

Values are expressed as mean ± SD of triplicates measurements. RV=Rambutan Vinegar, abcd = different superscripts signify significant mean differences at $p<0.05$ between concentration. efghi = different superscripts signify significant mean differences at $p<0.05$ between time.

4.4 Acetic Acid Content

Based on Table 4.11, there were significant ($p<0.05$) difference in the overall acetic acid content of Rose infused Rambutan Vinegar (RVR) under the influence of the concentration of Rose (grams) infused in Rambutan Vinegar (RV) and the time of infusion (weeks). The acetic acid content of all RVR were significantly different at $p<0.05$ with RV. The acetic acid content of RV(3 g Rose) increases significantly from Week 0 (6.27%) to Week 4 (7.40%) when compared to RV (5.67%). The acetic acid content of RV(1 g Rose) initially increases significantly and decreases significantly starting from Week 2 (5.07%) to Week 4 (2.27%) when compared to RV (5.67%). The

acetic acid content of RV(5 g Rose) increases significantly from Week 0 (6.47%) to Week 2 (6.40%) and decreases significantly starting from Week 3 (4.87%) to Week 4 (3.60%) when compared to RV (5.67%) The acetic acid content of RV(3 g Rose) increases significantly from Week 0 (6.27%) to Week 4 (7.60%) when compared to RV (5.67%).

The acetic acid content of all RVR were significantly different at $p<0.05$ starting from Week 1 to Week 4 in comparison with Week 0. The acetic acid content of RV(1 g Rose) and RV(5 g Rose) increases significantly at Week 1 and decreases significantly from Week 2 to Week 4 in comparison with Week 0. The acetic acid content of RV(3 g Rose) increases significantly from Week 1 (7.00%) to Week 4 (7.40%) in comparison with Week 0 (6.27%). The acetic acid content of RV decreases significantly starting from Week 1 to Week 2 and slightly increases at Week 3 and slightly decrease in Week 4 when compared to Week 0.

Table 4.11: Acetic Acid content of Rose infused Rambutan Vinegar under different formulations.

Week	Acetic Acid Content (%)			
	RV	RV (1 g Rose)	RV (3 g Rose)	RV (5 g Rose)
0	5.67 ± 0.12^{cf}	5.80 ± 0.20^{df}	6.27 ± 0.12^{af}	6.47 ± 0.23^{bf}
1	5.60 ± 0.00^{ce}	6.07 ± 0.12^{de}	7.00 ± 0.00^{ae}	7.47 ± 0.12^{be}
2	5.60 ± 0.00^{cf}	5.07 ± 0.12^{df}	7.33 ± 0.12^{af}	6.40 ± 0.00^{bf}
3	5.80 ± 0.00^{cg}	3.40 ± 0.00^{dg}	7.60 ± 0.00^{ag}	4.87 ± 0.12^{bg}
4	5.60 ± 0.00^{ch}	2.27 ± 0.12^{dh}	7.40 ± 0.00^{ah}	3.60 ± 0.00^{bh}

Values are expressed as mean ± SD of triplicates measurements. RV=Rambutan Vinegar, abcd = different superscripts signify significant mean differences at $p<0.05$ between concentration. efgh = different superscripts signify significant mean differences at $p<0.05$ between time.

Based on Table 4.12, there were significant ($p<0.05$) difference in the overall acetic acid content of Osmanthus infused Rambutan Vinegar (RVO) under the influence of the concentration of Osmanthus (grams) infused in Rambutan Vinegar (RV) and the time of infusion (weeks). The acetic acid content of all RVO were significantly different at $p<0.05$ with RV. The acetic acid content of RV(1 g Osmanthus) and RV(3 g Osmanthus) increases significantly from Week 0 to Week 3 and decreases significantly at Week 4 when compared to RV. The acetic acid content of RV(5 g Osmanthus) increases significantly starting from Week 0 (8.60%) to Week 4 (6.67%) when compared to RV (5.67%).

The acetic acid content of all RVO were significantly different at $p<0.05$ starting from Week 1 to Week 4 in comparison with Week 0. The acetic acid content of RV(1 g

Osmanthus), RV(3 g Osmanthus) and RV(5 g Osmanthus) decreases significantly starting from Week 1 to Week 4 in comparison with Week 0 whereas the pH of RV(1 g Osmanthus), RV(3 g Osmanthus) and RV(5 g Osmanthus) increases from Week 1 to Week 4. The relationship of acetic acid content and pH is inversely proportional. Therefore, the lower the acetic acid content, the higher the pH (low acidity) (Theapparat *et al.*, 2014). The continuous oxidation of produced acetic acid into carbon dioxide and water by *Acetobacter spp.* resulted in a decrease in acetic acid in fruit vinegars throughout fermentation (Raspor & Goranovi˘c, 2008).

Table 4.12: Acetic Acid content of Osmanthus infused Rambutan Vinegar under different formulations.

Week	Acetic Acid Content (%)			
	RV	RV (1 g Osmanthus)	RV (3 g Osmanthus)	RV (5 g Osmanthus)
0	5.67 ± 0.12^{de}	6.13 ± 0.12^{ce}	7.27 ± 0.23^{be}	8.60 ± 0.00^{ae}
1	5.60 ± 0.00^{df}	6.00 ± 0.20^{cf}	7.20 ± 0.00^{bf}	8.33 ± 0.12^{af}
2	5.60 ± 0.00^{dg}	5.80 ± 0.20^{cg}	6.60 ± 0.00^{bg}	7.80 ± 0.00^{ag}
3	5.80 ± 0.00^{dh}	5.80 ± 0.20^{ch}	6.20 ± 0.00^{bh}	7.40 ± 0.00^{ah}
4	5.60 ± 0.00^{di}	5.40 ± 0.00^{ci}	5.47 ± 0.12^{bi}	6.67 ± 0.12^{ai}

Values are expressed as mean ± SD of triplicates measurements. RV= Rambutan Vinegar, abcd = different superscripts signify significant mean differences at $p<0.05$ between concentration. efghi = different superscripts signify significant mean differences at $p<0.05$ between time.

4.5 Antioxidant Activity

Based on Table 4.13, there were significant ($p<0.05$) difference in the overall antioxidant activity of Rose infused Rambutan Vinegar (RVR) under the influence of the concentration of Rose (grams) infused in Rambutan Vinegar (RV) and the time of infusion (weeks). The antioxidant activity of all RVR were significantly different at $p<0.05$ with RV. The antioxidant activity of RV(3 g Rose) and RV(5 g Rose) increases significantly from Week 0 to Week 2 when compared to RV. When the concentration of a plant-based substance increases, antioxidant activity in terms of DPPH scavenging ability increases too (Lin *et al.,* 2020). The antioxidant activity of RV(1 g Rose) initially increases significantly at Week 0 (11.05%) and decreases significantly at Week 1 (-21.47%) and Week 2 (52.83%) when compared to RV (0.09%).

The antioxidant activity of all RVR were significantly different at $p<0.05$ starting from Week 1 to Week 2 in comparison with Week 0. The antioxidant activity of RV(1 g Rose) decreases significantly at Week 1 (-21.47%) and Week 2 (-52.83%) when compared with Week 0 (11.05%). RV(3 g Rose) decreases significantly at Week 1 (13.57%) and increases significantly at Week 2 (68.37%) when compared with Week 0 (16.62%). The antioxidant activity of RV(5 g Rose) increases significantly at Week 1 (52.29%) and Week 2 (70.53%) in comparison with Week 0 (29.83%). Based on the physicochemical properties (pH) of the RVR, the pH of RV(5 g Rose) increases over time which exceed the pH range of vinegar (pH 2.40 to pH 3.90) (Ousaaid *et al.*, 2022). Hence, RV(5 g Rose) was unstable and possibly a vinegar that undergo degradation in quality. Therefore, RV(3 g Rose) at Week 2 (68.37%) is the RVR with the highest antioxidant activity.

Table 4.13: Antioxidant Activity of Rose infused Rambutan Vinegar under different formulations.

Week	Antioxidant Activity (%)			
	RV	RV (1 g Rose)	RV (3 g Rose)	RV (5 g Rose)
0	0.09 ± 7.82^{cde}	11.05 ± 4.60^{cde}	16.62 ± 5.86^{bde}	29.83 ± 5.68^{ade}
1	-23.63 ± 2.62^{ce}	-21.47 ± 6.15^{ce}	13.57 ± 13.58^{be}	52.29 ± 9.10^{ae}
2	-17.07 ± 16.91^{cd}	-52.83 ± 4.28^{cd}	68.37 ± 8.11^{bd}	70.53 ± 13.21^{ad}

Values are expressed as mean ± SD of triplicates measurements. RV= Rambutan Vinegar, abc = different superscripts signify significant mean differences at $p<0.05$ between concentration. de = different superscripts signify significant mean differences at $p<0.05$ between time.

Based on Table 4.14, there were significant ($p<0.05$) difference in the overall antioxidant activity of Osmanthus infused Rambutan Vinegar (RVO) under the influence of the concentration of Osmanthus (grams) infused in Rambutan Vinegar (RV) and the time of infusion (weeks). The antioxidant activity of all RVO were significantly different at $p<0.05$ with RV. The antioxidant activity of RV(1 g Osmanthus), RV(3 g Osmanthus) and RV(5 g Osmanthus) increases significantly from Week 0 to Week 2 when compared to RV. *Osmanthus fragrans* displayed antioxidant activity when 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical-scavenging activity assay was conducted (Lee *et al.*, 2007).

The antioxidant activity of all RVO were not significantly different ($p>0.05$) starting from Week 1 to Week 2 in comparison with Week 0. Therefore, time of infusion is not a factor that affects the antioxidant activity in RVO. The RVO with the highest value of antioxidant activity is RV(1 g Osmanthus) at Week 2 (29.92%).

From Table 4.13 and Table 4.14, the antioxidant activity of RV(3 g Rose) at Week 2 (68.37%) is higher than RV(3 g Osmanthus) at Week 2 (0.18%) which is similar to the research conducted by Zheng *et al.* (2018) in which DPPH free radical scavenging activity of China rose is higher than Osmanthus flower. The antioxidant activity in Rose is higher than Osmanthus flower because red-coloured cultivars contains higher amount of polyphenols that contributes to the red-coloured anthocyanin pigments and exhibits antioxidant activity (Yoon and Kim, 2007).

Table 4.14: Antioxidant Activity of Osmanthus infused Rambutan Vinegar under different formulations.

Week	Antioxidant Activity (%)			
	RV	RV (1 g Osmanthus)	RV (3 g Osmanthus)	RV (5 g Osmanthus)
0	0.09 ± 7.82^{c}	6.02 ± 2.98^{b}	49.60 ± 7.34^{b}	80.05 ± 11.36^{a}
1	-23.63 ± 2.62^{c}	13.84 ± 7.44^{b}	11.23 ± 10.99^{b}	102.43 ± 16.45^{a}
2	-17.07 ± 16.91^{c}	29.92 ± 2.33^{b}	0.18 ± 13.88^{b}	124.44 ± 13.63^{a}

Values are expressed as mean ± SD of triplicates measurements. RV= Rambutan Vinegar, abc = different superscripts signify significant mean differences at $p<0.05$ between concentration. Tukey's Post Hoc test is not conducted for time of infusion of RVO because it is not significant.

4.6 Correlation between the Antioxidant Activity and Time of Infusion of the selected RVR and RVO

The correlation between the antioxidant activity and time of infusion of the selected Rose infused Rambutan Vinegar (RVR) and Osmanthus infused Rambutan Vinegar (RVO) was calculated using Pearson's correlation coefficient as shown in Table 4.15 and Table 4.16. Based on Table 4.15, the time of infusion of RV(3 g Rose) is positively and strongly correlated and is a significant correlation at $p<0.05$ with antioxidant activity in which ($r = 0.801$) and ($r^2 = 0.642$) that indicates 64% increase in antioxidant is due to the increase in time while the other 36% is based on other factors. Based on Table 4.16, the time of infusion RV(1 g Osmanthus) is positively and strongly correlated and is a significant correlation at $p<0.05$ with antioxidant activity in which ($r = 0.912$) and ($r^2 = 0.832$) that indicates 83% increase in antioxidant is due to the increase in time while the other 17% is based on other factors. According to Hajiaghaalipour *et al.* (2016), when the time of steeping (infusion) in a solvent increases, the antioxidant activity DPPH (%) inhibition increases too. Similarly, a study conducted by Pal *et al.* (2013) on the effect of infusion time of tea on the status of antioxidant state that the overall antioxidant and polyphenol content of tea infusions increased with time which supports the positive correlation between the antioxidant activity and time of infusion of RV(3 g Rose) and RV(1 g Osmanthus).

Table 4.15: Correlation between the Antioxidant Activity and Time of Infusion of RV(3 g Rose) Week 2

Variables	Time of Infusion	Antioxidant Activity
Time of Infusion	1	0.801*
Antioxidant Activity	0.801*	1

*. Correlation is significant at the 0.05 level (2-tailed)

Table 4.16: Correlation between the Antioxidant Activity and Time of Infusion of RV(1 g Osmanthus) Week 2

Variables	Time of Infusion	Antioxidant Activity
Time of Infusion	1	0.912*
Antioxidant Activity	0.912*	1

*. Correlation is significant at the 0.05 level (2-tailed)

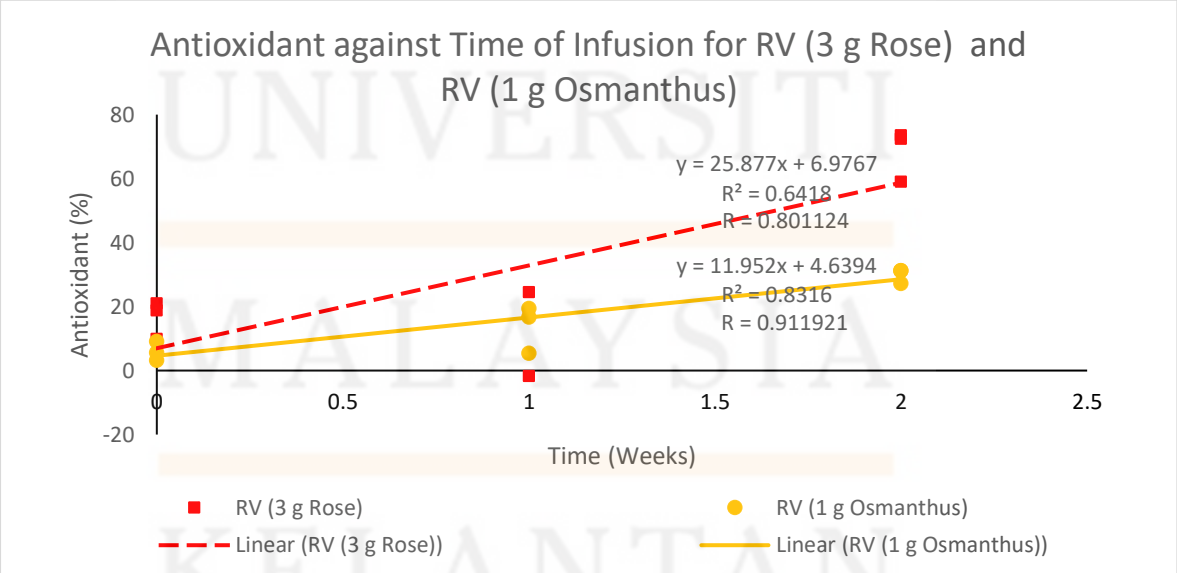

Figure 2.5: Antioxidant of edible flower infused rambutan vinegar against time of infusion.

4.7 Total Phenolic Content, Total Flavonoid Content and Anthocyanin pigment of the selected RVR and RVO

Total phenolic content of the RV(3 g Rose), RV(1 g Osmanthus) and RV is determined by using gallic acid standard curve range from 0 µg/mL to 250 µg/mL with equation :

$$y = 0.0065x + 0.338 \ (R^2 = 0.9852). \tag{4.1}$$

Total flavonoids content of the RV(3 g Rose), RV(1 g Osmanthus) and RV is determined by using quercetin standard curve range from 0.5 mg/mL to 5.0 mg/mL with equation :

$$y = 0.0868x - 0.0907 \ (R^2 = 0.9862). \tag{4.2}$$

Based on Table 4.17, there were significant ($p<0.05$) difference in the total phenolic content (TPC), total flavonoid content (TFC) and anthocyanin pigment of Rose infused Rambutan Vinegar (RVR) and Osmanthus infused Rambutan Vinegar (RVO) when compared to RV. Based on Table 4.17, the TPC of RV(3 g Rose) at Week 2 (152.29 µg GAE/mL) and RV(1 g Osmanthus) at Week 2 is higher than the TPC of RV (-27.19 µg GAE/mL). The TPC of RV shows negative values because the antioxidant activity of RV also shows negative value. This is because the relationship of antioxidant activity and TPC is directly proportional. The TPC of RV is negative because it was exposed to factors

such as temperature, light and oxidation which caused degradation of the phenolic compounds in RV (Li *et al.*, 2012). When comparing the TPC among RVR and RVO, the TPC of RV(3 g Rose) at Week 2 (152.29 µg GAE/mL) has the highest value when compared to RV(1 g Osmanthus) at Week 2 (68.82 µg GAE/mL). According to Cendrowski *et al.* (2017), polyphenolic compounds including phenolic acid, flavonols, flavan-3-ols, and ellagitannins are present in Rose. Based on the study conducted by Li *et al.* (2017), the concentration of total phenolic compounds in *Osmanthus fragrans* flowers is higher than the calculated phenolic content in regularly consumed legumes and cereals. Moreover, Gonçalves *et al*. (2020) has determined that Roses exhibits the highest TPC when methanol is used as a solvent for extraction. Based on a research conducted by Zheng *et al.* (2018), the TPC of Rose is higher than the TPC of Osmanthus flower. According to Kaissoon *et al.* (2011), the total phenolic content varied among different plants species (2011). Based on the literature, Roselló-Soto *et al*. (2019) state that acidic conditions (low pH) extract higher amount of phenolic compounds because acidic condition increased the interaction of phenolic compounds with the solvent. Therefore, vinegar which is in the pH range of pH 2.40 to pH 3.90 is able to extract high amounts of phenols (Ousaaid *et al*., 2022).

Besides, flavonoids are a large and diversified class of natural compounds that are the most essential natural phenolics (Prasad *et al.*, 2009). The TFC in RV(3 g Rose) at Week 2 (1.67 mg QE/mL) and RV(1 g Osmanthus) at Week 2 (1.79 mg QE/mL) is higher than the TFC of RV (0.14 mg QE/mL). When comparing the TFC values among RVR and RVO, the TFC in RV(1 g Osmanthus) at Week 2 (1.79 mg QE/mL) is higher than the TFC of RV(3 g Rose) at Week 2 (1.67 mg QE/mL). This result was the comparable with the findings from Zheng *et al.* (2018) which states the TFC in Osmanthus is the highest when compared with the TFC of other top 10 edible flowers in China such as

honeysuckle, chrysanthemum, nasturtium, China rose and others. Based on a research conducted by (Song *et al.*, 2021), *Osmanthus fragrans* has a lot of phenolic compounds, such as lignan and flavonoids, which are powerful antioxidants. The extraction efficiency of flavonoids is affected by the pH of solvent because the at different pH, the ionic strength of the flavonoid compound is altered which affects the solubility of the flavonoid compounds (Chaves *et al.*, 2020). A recent study conducted by Mai *et al.* (2020), it was discovered that the pH of solvent affects the recovery of flavonoids from *Euonymus alatus* (Burning bush) in which the recoveries of flavonoid is higher in acidic conditions (pH 2.5 to pH 3.5) and lower at higher pH.

Furthermore, anthocyanins play a significant function in flowers, not only because of their colour, but also because of their antioxidant properties (Gonçalves *et al.*, 2020). Anthocyanin is one of the subclasses of flavonoids which are natural pigments that give flowers their appealing colour in shades of orange, red, pink, and blue (Pires *et al.*, 2021). Based on Table 4.17, the anthocyanin pigment content in RV(3 g Rose) at Week 2 (1.89 mg C3G/L) and RV(1 g Osmanthus) at Week 2 (0.78 mg C3G/L) is higher than the TFC of RV (0.22 mg C3G/L). When comparing the anthocyanin pigment content among RVR and RVO, the anthocyanin pigment content in RV(3 g Rose) at Week 2 (1.89 mg C3G/L) is higher than the anthocyanin pigment content in RV(1 g Osmanthus) at Week 2 (0.78 mg C3G/L). Delphinidin-3-o-glucoside (dlp-3-oglu), cyanidin-3-o-glucoside (cyd-3-o-glu), pelargonidin-3-o-glucoside (plg3-o-glu) and malvidin-3-o-glucoside (mlv-3-o-glu) are the most common components of anthocyanins (water-soluble vacuolar pigment) (Karaaslan-Ayhan & Yaman, 2021). High number of anthocyanins is extracted by using methanol while the use of water as solvent extract the least number of anthocyanins (Karaaslan-Ayhan & Yaman, 2021). Based on the literature, Karaaslan-Ayhan & Yaman (2021) show that the most anthocyanin extracted by solvent water was pelargonidin-3-o-

glucoside (plg3-o-glu) followed by cyanidin-3-o-glucoside (cyd-3-o-glu). Based on Table 4.17, the anthocyanin pigment content in RV(3 g Rose) at Week 2 (1.89 mg C3G/L) is higher than the anthocyanin pigment content in RV(1 g Osmanthus) at Week 2 (0.78 mg C3G/L) because, Rose and Osmanthus contains cyanidin-3-o-glucoside (cyd-3-o-glu) (Luo *et al.*, 2019; Wan *et al.*, 2019). There is also a possibility of the presence of pelargonidin-3-o-glucoside (plg3-o-glu) in RV(3 g Rose) because solvent water extracts pelargonidin-3-o-glucoside (plg3-o-glu) and this anthocyanin colour provides red pigment which is present in Rose (Wan *et al.*, 2019).

Table 4.17: Total Phenolic Content, Total Flavonoid Content and Anthocyanin Pigment of the selected Rose infused Rambutan Vinegar and Osmanthus infused Rambutan Vinegars.

Sample	Total Phenolic Content (µg GAE/mL)	Total Flavonoid Content (mg QE/mL)	Anthocyanin Pigment (mg C3G/L)
RV	-27.19 ± 2.65^{c}	0.14 ± 0.05^{c}	0.22 ± 0.10^{c}
RV (3 g Rose) Week 2	152.29 ± 14.99^{a}	1.67 ± 0.02^{b}	1.89 ± 0.10^{a}
RV (1 g Osmanthus) Week 2	68.82 ± 10.38^{b}	1.79 ± 0.02^{a}	0.78 ± 0.10^{b}

Values are expressed as mean ± SD of triplicates measurements. RV= Rambutan Vinegar, abc = different superscripts signify significant mean differences at $p<0.05$ between concentration.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In conclusion, concentration of flower infused in Rambutan Vinegar and time of infusion plays an important role in the physicochemical properties and antioxidant activity of RVR and RVO. The optimum concentration of flower infused in 5% Rambutan Vinegar and time of infusion for RVR is RV(3 g Rose) at Week 2 and for RVO is RV(1 g Osmanthus) at Week 2. The active ingredients and properties of 5% Rambutan Vinegar were enhanced through the infusion of flower in Rambutan Vinegar. The time of infusion of RVR and RVO should not exceed 2 weeks, hence, the ideal time of infusion would be within 1 to 14 days. Rambutan Vinegar is also a good solvent to extract phenolic compounds because phenols dissolve in acidic conditions. Flavonoid compounds and anthocyanin pigment was also extracted by Rambutan Vinegar which shows improvement in the colour of 5% Rambutan Vinegar. Total phenolic content, total flavonoid content and anthocyanin pigment is directly proportional to the antioxidant activity of flower infused Rambutan Vinegar.

5.2 Recommendations

The recommendations for future works would include sensory evaluation, stability test and microbiological analysis, carotenoid analysis and the identification of bioactive compounds to be conducted on both Rose infused Rambutan Vinegar (RVR) and Osmanthus infused Rambutan Vinegar (RVO). Since flavonoid was extracted by using Rambutan Vinegar, sensory evaluation could be conducted to determine the acceptability on the taste of RVR and RVO. Moreover, carotenoid analysis is more suitable for pigment analysis of RVO because the anthocyanin pigment spectrum is more to Rose. Stability test could also be conducted to if the quality of RVR and RVO is affected over time by various environmental factors. Microbiological analysis could also be conducted to determine the presence of acetic acid bacteria in RVR and RVO that changes over time. Besides, other types of edible flower could also be infused into Rambutan Vinegar to investigate its suitability with Rambutan Vinegar in order to increase the type of flower flavoured vinegar in the Malaysian market.

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APPENDICES

A) SPSS Data of RVR Lightness

Tests of Between-Subjects Effects

Dependent Variable: Lightness of Rose

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3755.923[a]	19	197.680	125.977	.000
Intercept	214251.553	1	214251.553	136537.995	.000
Concentration	1438.743	3	479.581	305.627	.000
Time	1017.822	4	254.456	162.159	.000
Concentration * Time	1299.358	12	108.280	69.004	.000
Error	62.767	40	1.569		
Total	218070.243	60			
Corrected Total	3818.690	59			

B) SPSS Data of RVR Redness

Tests of Between-Subjects Effects

Dependent Variable: Redness of Rose

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	875.507[a]	19	46.079	2512.731	.000
Intercept	1218.062	1	1218.062	66421.626	.000
Concentration	314.718	3	104.906	5720.582	.000
Time	274.975	4	68.744	3748.641	.000
Concentration * Time	285.814	12	23.818	1298.799	.000
Error	.734	40	.018		
Total	2094.302	60			
Corrected Total	876.240	59			

C) SPSS Data of RVR Yellowness

Tests of Between-Subjects Effects

Dependent Variable: Yellowness of Rose

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3343.796[a]	19	175.989	1373.057	.000
Intercept	12950.410	1	12950.410	101038.258	.000
Concentration	1576.509	3	525.503	4099.941	.000
Time	1509.551	4	377.388	2944.354	.000
Concentration * Time	257.736	12	21.478	167.570	.000
Error	5.127	40	.128		
Total	16299.334	60			
Corrected Total	3348.923	59			

D) SPSS Data of RVR pH

Tests of Between-Subjects Effects

Dependent Variable: pH of Rose

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	13.282[a]	19	.699	699.044	.000
Intercept	834.028	1	834.028	834028.167	.000
Concentration	3.366	3	1.122	1121.944	.000
Time	4.948	4	1.237	1236.917	.000
Concentration * Time	4.968	12	.414	414.028	.000
Error	.040	40	.001		
Total	847.350	60			
Corrected Total	13.322	59			

E) SPSS Data of RVR TSS

Tests of Between-Subjects Effects

Dependent Variable: TSS of Rose

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	38.659[a]	19	2.035	763.010	.000
Intercept	519.204	1	519.204	194701.563	.000
Concentration	17.188	3	5.729	2148.562	.000
Time	8.838	4	2.210	828.594	.000
Concentration * Time	12.632	12	1.053	394.760	.000
Error	.107	40	.003		
Total	557.970	60			
Corrected Total	38.766	59			

F) SPSS Data of RVR Acetic Acid Content

Tests of Between-Subjects Effects

Dependent Variable: TCA of Rose

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	114.199[a]	19	6.010	601.049	.000
Intercept	1992.961	1	1992.961	199296.067	.000
Concentration	50.978	3	16.993	1699.267	.000
Time	24.049	4	6.012	601.233	.000
Concentration * Time	39.172	12	3.264	326.433	.000
Error	.400	40	.010		
Total	2107.560	60			
Corrected Total	114.599	59			

G) SPSS Data of RVR Antioxidant Activity

Tests of Between-Subjects Effects

Dependent Variable: Antioxidant of rose

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	48446.094[a]	11	4404.190	52.576	.000
Intercept	5427.969	1	5427.969	64.797	.000
Concentration	33238.885	3	11079.628	132.265	.000
Time	953.765	2	476.883	5.693	.009
Concentration * Time	14253.444	6	2375.574	28.359	.000
Error	2010.447	24	83.769		
Total	55884.511	36			
Corrected Total	50456.542	35			

H) SPSS Data of RVR Antioxidant Activity

Tests of Between-Subjects Effects

Dependent Variable: Antioxidant of Osmanthus

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	75280.434[a]	11	6843.676	59.660	.000
Intercept	35549.019	1	35549.019	309.902	.000
Concentration	66498.393	3	22166.131	193.235	.000
Time	537.352	2	268.676	2.342	.118
Concentration * Time	8244.689	6	1374.115	11.979	.000
Error	2753.056	24	114.711		
Total	113582.508	36			
Corrected Total	78033.489	35			

I) SPSS Data of RVO Lightness

Tests of Between-Subjects Effects

Dependent Variable: Lightness of Osmanthus

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1850.711[a]	19	97.406	53.057	.000
Intercept	279426.423	1	279426.423	152203.541	.000
Concentration	631.507	3	210.502	114.661	.000
Time	856.861	4	214.215	116.683	.000
Concentration * Time	362.344	12	30.195	16.447	.000
Error	73.435	40	1.836		
Total	281350.569	60			
Corrected Total	1924.146	59			

J) SPSS Data of RVO Redness

Tests of Between-Subjects Effects

Dependent Variable: Redness of Osmanthus

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	126.757[a]	19	6.671	1593.498	.000
Intercept	53.167	1	53.167	12699.006	.000
Concentration	97.268	3	32.423	7744.299	.000
Time	15.544	4	3.886	928.195	.000
Concentration * Time	13.945	12	1.162	277.565	.000
Error	.167	40	.004		
Total	180.091	60			
Corrected Total	126.925	59			

K) SPSS Data of RVO Yellowness

Tests of Between-Subjects Effects

Dependent Variable: Yellowness of Osmanthus

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1922.765[a]	19	101.198	3132.423	.000
Intercept	12371.140	1	12371.140	382928.396	.000
Concentration	1763.554	3	587.851	18195.979	.000
Time	22.292	4	5.573	172.503	.000
Concentration * Time	136.919	12	11.410	353.175	.000
Error	1.292	40	.032		
Total	14295.197	60			
Corrected Total	1924.057	59			

L) SPSS Data of RVO pH

Tests of Between-Subjects Effects

Dependent Variable: pH of Osmanthus

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3.297[a]	19	.174	80.097	.000
Intercept	710.016	1	710.016	327699.692	.000
Concentration	2.588	3	.863	398.154	.000
Time	.112	4	.028	12.962	.000
Concentration * Time	.597	12	.050	22.962	.000
Error	.087	40	.002		
Total	713.400	60			
Corrected Total	3.384	59			

M) SPSS Data of RVO TSS

Tests of Between-Subjects Effects

Dependent Variable: TSS of Osmanthus

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	16.289[a]	19	.857	171.467	.000
Intercept	672.011	1	672.011	134402.133	.000
Concentration	12.855	3	4.285	856.978	.000
Time	2.356	4	.589	117.800	.000
Concentration * Time	1.079	12	.090	17.978	.000
Error	.200	40	.005		
Total	688.500	60			
Corrected Total	16.489	59			

N) SPSS Data of RVO Acetic Acid content

Tests of Between-Subjects Effects

Dependent Variable: TCA of Osmanthus

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	56.029[a]	19	2.949	245.743	.000
Intercept	2493.571	1	2493.571	207797.556	.000
Concentration	41.229	3	13.743	1145.259	.000
Time	9.549	4	2.387	198.944	.000
Concentration * Time	5.251	12	.438	36.463	.000
Error	.480	40	.012		
Total	2550.080	60			
Corrected Total	56.509	59			

O) Correlation between Antioxidant activity and Time of infusion of RVR

Correlations

		Time of Rose 3g	Antioxidant of Rose 3g
Time of Rose 3g	Pearson Correlation	1	.801**
	Sig. (2-tailed)		.009
	N	9	9
Antioxidant of Rose 3g	Pearson Correlation	.801**	1
	Sig. (2-tailed)	.009	
	N	9	9

P) Correlation between Antioxidant activity and Time of infusion of RVO

Correlations

		Time of Osmanthus 1g	Antioxidant of Osmanthus 1g
Time of Osmanthus 1g	Pearson Correlation	1	.912**
	Sig. (2-tailed)		.001
	N	9	9
Antioxidant of Osmanthus 1g	Pearson Correlation	.912**	1
	Sig. (2-tailed)	.001	
	N	9	9

Q) SPSS data of Total Phenolic Content of RV(3 g Rose) at Week 2 and RV(1 g Osmanthus) at Week 2

ANOVA

Total Phenolic Content

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	48396.140	2	24198.070	213.827	.000
Within Groups	679.000	6	113.167		
Total	49075.141	8			

R) SPSS data of Total Flavonoid Content of RV(3 g Rose) at Week 2 and RV(1 g Osmanthus) at Week 2

ANOVA

Total Flavonoid Content

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.072	2	2.536	2835.384	.000
Within Groups	.005	6	.001		
Total	5.077	8			

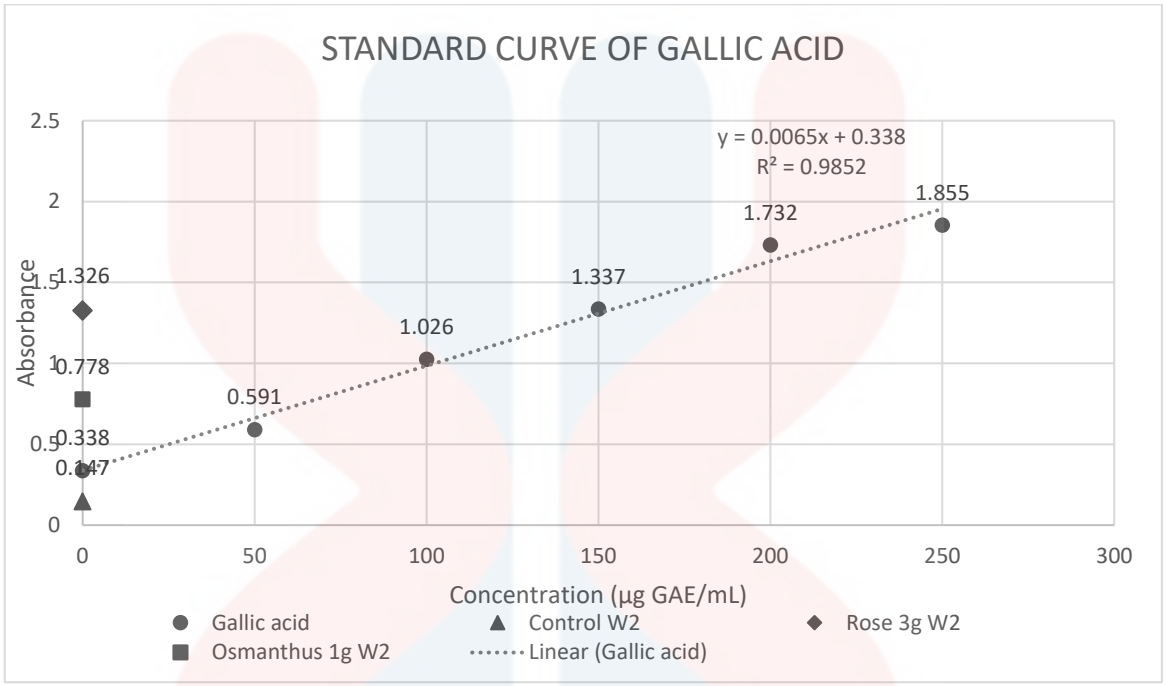
S) SPSS data of Anthocyanin pigment of RV(3 g Rose) at Week 2 and RV(1 g Osmanthus) at Week 2

ANOVA

Monomeric Anthocyanin Pigment

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.338	2	2.169	233.333	.000
Within Groups	.056	6	.009		
Total	4.393	8			

T) Calibration curve of gallic acid standard for total phenolic content determination.



U) Calibration curve of quercetin standard for total flavonoid content determination.