UNDERGRADUATE THESIS

ANALYSIS OF FERMENTATION TIME VARIATIONS ON pH LEVEL AND ANTIOXIDANT ACTIVITY OF SYNBIOTIC DRINK OF MANGOSTEEN RIND (*Garcinia mangostana L.*)

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DEPARTMENT OF NUTRITION
FACULTY OF HEALTH
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Submitted to Undegraduate Program University of Darussalam Gontor in Partticial Fulfillment of The Requirments for Health Science

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PONOROGO

2019

UNIDA

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UNIVERSITY OF DARUSSALAM GONTOR

ANALISIS VARIASI WAKTU FERMENTASI TERHADAP NILAI pH DAN AKTIFITAS ANTIOKSIDAN MINUMAN SINBIOTIK KULIT MANGGIS (Garcinia mangostana L.)

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ABSTRAK

Latar Belakang: Minuman sinbiotik kulit manggis adalah salah satu produk fermentasi bakteri asam laktat. Bakteri probiotik yang digunakan dalam penelitian ini adalah bakteri *Lactobacillus casei*. Lama fermentasi merupakan salah satu faktor yang mempengaruhi kualitas minuman fermentasi, terutama minuman sinbiotik. **Tujuan:** Penelitian ini bertujuan untuk menganalisis pengaruh waktu fermentasi terhadap nilai pH dan aktivitas antioksidan dari minuman sinbiotik kulit manggis. Metode: Metode penelitian menggunakan Rancangan Acak Kelompok (RAK) dengan faktor fermentasi yang lama (12 jam, 24 jam, 36 jam, dan 48 jam). Variabel diukur dengan pH meter untuk nilai pH dan metode DPPH untuk aktivitas antioksidan dengan tiga replikasi. Data yang diperoleh dianalisis menggunakan ANOVA dilanjutkan dengan uji Tukey pada tingkat kepercayaan 5%. **Hasil:** Berdasarkan hasil penelitian, nilai pH terendah adalah 3,08 pada fermentasi 48 jam. Nilai pH tertinggi adalah 4,24 pada fermentasi 12 jam. Aktivitas antioksidan terlemah adalah 160,293 ppm pada 12 jam, dan aktivitas antioksidan terkuat adalah 92,265 ppm pada 48 jam. Sampel terbaik dilihat dari tingkat pH dan aktivitas antioksidan yang diperoleh adalah minuman sinbiotik dengan waktu fermentasi 24 jam. Waktu fermentasi berpengaruh signifikan terhadap nilai pH dan aktivitas antioksidan. **Kesimpulan:** Semakin lama fermentasi, nilai pH menurun dan tingkat aktivitas antioksidan meningkat.

Kata kunci: *aktivitas antioksidan, kulit manggis, pH, minuman sinbiotik*

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ABSTRACT

Backgound: Synbiotic drink of mangosteen rind is one of the lactic acid bacteria fermentation products. The probiotic bacteria used in this study were *Lactobacillus casei* bacteria. The duration of fermentation is one of the factors that influences the quality of the fermented drink, especially synbiotic drinks. **Objective:** This study aims to analyze the effect of fermentation time on pH values and antioxidant activity of synbiotic drink of mangosteen rind. **Method:** The research method used Randomized Block Design (RBD) with long fermentation factors (12 hours, 24 hours, 36 hours, and 48 hours). The variable was measured by pH meter for pH level and DPPH method for antioxidant activity with three replications. The data obtained were analyzed using ANOVA continued with the Tukey test at the level of 5%. **Result:** Based on the results of the study, the lowest pH value was 3,08 at 48 hours fermentation. The highest pH value was 4,24 at 12 hours fermentation. The weakest antioxidant activity was 160,293 ppm at 12 hours, and the strongest antioxidant activity was 92,265 ppm at 48 hours. The best sample from the pH level and antioxidant activity obtained was a synbiotic drink with a 24-hour fermentation time. Fermentation time has significantly affected on pH value and antioxidant activity. **Conclusion:** The longer fermentation, the more pH levels decreased and antioxidant activity levels increased.

Keywords: *antioxidant activity, mangosteen rind, pH, synbiotic drink*

DECLARATION

I hereby,

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Title	: **Analysis of Fermentation Time Variations on pH Level and Antioxidant Activity of Synbiotic Drink of Mangosteen Rind (*Garcinia mangostana L.*)**

I sincerely declare that this thesis initially belongs to my work and not belong to another researcher for a different degree. Furthermore, this thesis is never published before, except for some parts with their original references.

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Ponorogo, March 28st 2019 M
Rajab 21th 1440 H

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UNDERGRADUATE THESIS

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ACKNOWLEDGEMENTS

Praise and deep gratitude to Allah SWT for the abundance of grace, and guidance of Him given to the writer that made this thesis can be completed properly. Greetings and shalawat may always be devoted to the Prophet Muhammad SAW.

This thesis is entitled “Analysis of Fermentation Time Variations on pH Level And Antioxidant Activity of Synbiotic Drink of Mangosteen Rind (*Garcinia mangostana L.*)". This was compiled as one of the conditions for obtaining a bachelor's degree at the Department of Nutrition, Faculty of Health Sciences, University f Darussalam Gontor.

In writing this thesis, the author gets help and support from many parties, both directly and indirectly, in the form of motivation, thoughts, and instructions so that this thesis can be resolved as it should.

The highest appreciation and gratitude from the author goes to both of my beloved parents, my Mother and Father who have given their prayers and motivation and support both in the form of material and advice, so that this final assignment can be completed well. Because of His love and guidance, my brothers and sisters, as well as the entire extended family of writers who cannot be mentioned one by one, thank you for your prayers, love and guidance to the author, there is no proper word to express how much love and compassion you have given me. They are the greatest enthusiasm for the writer to complete this thesis. May Allah SWT always give His grace and protection to you.

The author does not forget to thank as much as possible through expression of happiness to:

1. Prof. Dr. KH Amal Fathullah Zarkasyi, M.A., as Rector of the University of Darussalam who has given me the opportunity to complete my studies at University of Darussalam Gontor,
2. drg. Ruskiah Octavia, M.M., as Dean of the Faculty of Health Sciences, University of Darussalam Gontor,

3. Amilia Yuni Damayanti, S. Gz, M. Gizi., As Deputy Dean I, Health Sciences Faculty, University of Darussalam Gontor,
4. Fathimah, S. Gz, MKM., as Head of the Department of Nutrition, Faculty of Health Sciences, University of Darussalam Gontor,
5. Mira Dian Naufalina, S. Gz, M. Gizi., As the first advisor who has spent her time and thoughts in guiding the author in completing this thesis and providing guidance and direction in correcting all the shortcomings of this thesis,
6. Dianti Desita Sari, S. Pt, M. Si., As the second advisor who has spent her time and thoughts in guiding the author in completing this thesis and providing guidance and direction in correcting all the flaws in this thesis,
7. Joyeti Darni, S. Gz, M. Gizi., As the examiner who has given many inputs and suggestions for the perfection of this thesis,
8. Mr., Mrs. Lecturers, and all Nutrition Department Staff sfor the outpouring of knowledge and all the assistance given to the author since taking Nutrition education to date,
9. Friends of Arms of 2015 who always pray, give motivation and a variety of useful information to the author to be able to complete this scientific thesis,
10. All parties involved are either directly or indirectly in this research which without reducing respect cannot be mentioned by the author one by one.

The author realizes that this thesis still has many weaknesses. But the hope is that it can be useful for further research, especially in the field of nutrition and hopefully it is worth the worship beside Allah SWT. Amin Ya Rabbal Alamin.

Ponorogo, March 28th, 2019

Miftahul Ni'maturrohmi

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CHAPTER I

INTRODUCTION

1.1 Background of Research

Free radicals are highly reactive compounds in the body because the molecule has no spouse. Free radicals exposure cannot be separated from daily life. Pollution from factories, vehicles, cigarettes, alcohol, and food are some of the sources of free radicals. Based on Riskesdas 2018 data, the proportion of tobacco consumption aged 15 and over has increased 1% from 32.8 at Sirkesnas 2016 in which the data is 33.8 on Riskesdas 2018 . On the proportion of alcoholic drinks, the Riskesdas data on 2018 shows 3.3 which means an increase from 2007 which has a total alcoholic 3.0 (Kemenkes, 2018). Baked and fried foods are one of the sources of free radicals from food (Kahkeshani et al, 2015).

Some degenerative diseases are caused by free radicals. Reactive and unstable free radicals cause damage to living cells. Cell function is not optimal due to damage to living cells causing long-term degenerative diseases (Sutrisna, 2013). WHO data shows that in 2008 55% from 14.5 million deaths in South Asia are caused by degenerative disease (WHO, 2011). Another study concluded that antioxidants have therapeutic value against degenerative disease (Barhe and Tchouya, 2014).

Antioxidants are compounds that can counteract and reduce free radicals activity by providing electrons to pair up with the free radicals compounds (Halliwell, 2012). There are two groups of antioxidants, i.e. natural antioxidant which is an antioxidant derived from the natural ingredients potentially preventing free radicals and antioxidant synthetic derived from chemicals (Isfahlan, 2010). The human body has an antioxidant which reserves slightly, so antioxidants from outside the body are needed to ward off radicals when the body is exposed to free radicals. The sources of natural antioxidants are derived from vegetables and fruit (Otsuki et al,

2010). Based on Riskesdas 2018 data, the proportion of consumption of fruit or vegetables lack in Indonesia has increased 2% where at Riskesdas 2013 it was 93.5% increased, to 95.5% in 2018 (Kemenkes, 2018). This is one of the reason that Indonesian people need innovation to consume sources of antioxidants, one of the ways is by using a synbiotic drink of mangosteen rind.

Mangosteen is a fruit that its utilization are still managed in a simple utilization despite the increased market potential, which some countries have long used the mangosteen fruit as a medicinal and therapeutic agents, especially the rind part (Permana, 2010). Mangosteen fruit is a plant that originated from Southeast Asia includes Indonesia, Malaysia, Thailand, and Myanmar. The mangosteen fruit is generally addressed by " Queen of Fruits " because the mangosteen fruit contains beneficial nutrient for preventing and treating various diseases such as cancer, heart disease, arthritis, diarrhea, tonsillitis, vaginal discharge, and dysentery. In addition to the utilization of the fruit, the rind is also widely used in the field of health. Mangosteen rind acts as an anti-hypertension, anti-cancer, anti-inflammatory, anti-diabetic and anti–HIV (Nugroho, 2012). Mangosteen rind which is the largest part of the mangosteen fruit is categorized as residue. Mangosteen rind contains active compounds known as xanthones. Xanthone has a role as a powerful compared to vitamin C and vitamin E to counteract free radicals, preventing cell damage, and inhibiting cell degeneration (Mardiana, 2012).

Allah says in Al - Qur ' an Surah Asy- Syu'araa : 80 :

وَإِذَا مَرِضْتُ فَهُوَ يَشْفِينِ (٠٨)

"And when I sick, then He health me"

The verse above explains that Allah has given all diseases to humans with the bidders and will be cured by permission of Allah. The mangosteen rind was one of the preventive agents of some diseases especially cancer because it contains antioxidant.

Synbiotic is a combination of probiotics and prebiotics (Yui, 2006). One of the agricultural commodities that contains prebiotics is mangosteen. Synbiotic drink products are developed with a dairy-based carrier because the high sugar content can be utilized by probiotics and prebiotics in the mangosteen rind so that the use of mangosteen rind as a prebiotic and *L. casei* as probiotics can produce the synbiotic drink.

Processed products and patent research about mangosteen continuesto grow such as juice, puree, concentrates, food supplement, herbal remedies and cosmetics. Mangosteen rind products have a good market prospects. Japan already developed products containing extracts of Panaxathone xanthone blend (80% α-mangostin and 20% γ-mangostin) used in breast cancer chemoterapy (Doi, 2009). Currently, in Indonesia processed products of mangosteen rind are developing such as mangosteen drink in the form of the juice using mangosteen, Mangosteen rind flour in a bag (powder bag), Mangosteen rind crumbs in capsules. The level of safety and hedonics for some ready to use commercial products cannot be guaranteed such as mangosteen rind flour in capsules which is risky for health because it still contains sap and other components that cannot be digested by the body (Permana, 2012).

The natural taste of the mangosteen rind extract is bitter when consumed since it scaused by groups of phenols compounds, xanthone compounds, including anthocyanin or tannin/catechins (Oliveira et al, 2014). Fermentation process in the making of sinbiotic drink may improve the taste and cover the bitter taste of mangosteen rind. Fermentation process leads to the increases of nutritional value of the macronutrient, micronutrient and antioxidant level on synbiotic drink of mangosteen rind (Pamungkas, 2011).

This study was designed to answer the problem lies if there are differences in antioxidant activity and pH levels of each sample with different fermentation times. Expected result are to improve the quality of life of the communities that use local natural resources, increasing the economic value of tropical fruit mangosteen particularly high in antioxidants and provide

additional insight into the potential of local materials in the environment that can be used for the treatment and prevention of degenerative diseases.

1.2 Statement of the Problems

1. How does the effect of fermentation time on pH level of synbiotic drink of mangosteen rind?
2. How does the effect of fermentation time on antioxidant activity of synbiotic drink of mangosteen rind?

1.3 Objectives of Research

1.3.1 General Objective

Analyzing pH level and antioxidant activity of synbiotic drink of mangosteen rind with variations of fermentation time

1.3.2 Special Objectives

a. Analyzing the effect of fermentation time on pH level of synbiotic drink of mangosteen rind
b. Analyzing the effect of fermentation time on antioxidant activity of synbiotic drink of mangosteen rind

1.4 Benefits of Research

1.4.1 Theoretical Benefits

a. Giving information ON the pH level and antioxidant activity of synbiotic drink of mangosteen rind
b. Becoming a reference in subsequent studies
c. Providing ideas and inputs if there are similar studies
d. Improving the ability of the author to use laboratory equipments

1.4.2 Practical Benefits

a. Giving information about the pH level and antioxidant activity of synbiotic drink of mangosteen rind as a functional food

b. Improving the economic value of the mangosteen rind

1.5 Authenticity and Formers Reasearch

Table 1. 1 Authencity and Former Research

Researcher's Name	Title	Research Findings
W Asep Permana, Siti Mariana Widayanti, Sulusi Prabawati, Dondy A Setyabudi (2012)	Sifat Antioksidan Bubuk Kulit Buah Manggis (*Garnicia mangostana L.*) Instan dan Aplikasinya Untuk Minuman Fungsional Berkarbonasi	Mangosteen pericarp instant powder contained high levels of alpha-mangostin of 0.59 mg / g, anthocyanin 1.13 mg / g and phenolic content of 8.49 mg / g dry sample weight units. Antioxidant capacity amounted to 19.72 mg / g.
Stevi G, Dungir, Dewi G. Katja, Vanda S. Kamu (2012)	Aktivitas Antioksidan Ekstrak Fenolik dari Kulit Buah Manggis (*Garnicia mangostana L.*)	The highest content of phenolic compounds is in the methanol of dried samples (MK), followed by methanol of wet samples (MB), water of dried samples (AK), and the latter water wet sample (AB). The antioxidant activity is in MK is 44.49 mg / L, followed by premises MB, AK, AB with a value of 54.95; 346.73; 346.74 mg / l.

Researcher's Name	Title	Research Findings
Arum Lulu Mawar, Nur Aini, Gunawan Wijonarko (2018)	Formulasi Minuman Synbiotic dari Susu dan Ubi Jalar Menggunakan *Lactobacillus casei*	Synbiotic drink with the best formula is the percentage of *Lactobacillus casei* 2% and the ratio of sweet potato juice: skim milk (3: 1) The results are of the pH value 5.6, total dissolved solids 26o Brix, a total of 109 CFU of probiotics 1.56x, resistant to low pH 337.4%.
Suharyono A.S, Fibra Nurainy, Samsul Rizal, M. Kurniadi (2012)	L. *casei* Growth on Various Fermentation Time Synbiotic Drink of Green Cincau Extract (*Peremna oblongifolia merr*)	The result of obtained observation was examined its homogeneity with Bartlett test and growing data with Tuckey test, then the data were analyzed its heterogeneity to know the presence of difference between 1% and 5% of Less Significant Differential. The result showed that the appropriate and optimal duration of fermentation to produce a synbiotic drink of green cincau leaves extract was 16 hours with product characteristic possess the highest total amount of LAB at 1.78x1010 CFU/ml with pH 3.40 and acid total 3.30%.

The authenticity of the research is based on several previous studies which have relatively similar characteristics in terms of research themes, although they differ in terms of the criteria of the subjects and variables studied. The research will be conducted on the characteristics of the synbiotic drink of mangosteen rind. Research related to mangosteen rind were conducted by Permana et al (2012) who made carbonated functional

drink products and research that have been conducted by Dungir (2012) under the title "Antioxidant Activity of Phenolic Extract from Mangosteen Rind (*Garcinia mangostana L.*)". The similarity of the research conducted by Permana et al (2012) and Dungir (2012) with the research that the researcher did was using the same ingredients of mangosteen rind and testing variables for antioxidant activity, while the differences were in the processing of mangosteen rind and the variables studied. Permana et al (2012) processes mangosteen rind into carbonated drink and Dungir (2012) by using mangosteen rind phenolic extract to study, while the researcher processes mangosteen rind into a synbiotic drink and research variables not only for antioxidant activity but also pH levels.

Another research is the formulation of synbiotic drink from milk and sweet potatoes using *Lactobacillus casei* (Mawar, 2018). The similarity of research conducted by Mawar with the researchers is in processing basic ingredients by making the synbiotic drink, in which the similarities in the use of lactic acid bacteria namely *Lactobacillus casei* and the similarity of research variables namely testing pH levels. The difference in the research is in the basic ingredients of the product which in the Arum's study the manufacture of sweet potato-based synbiotic drink, while in this study the manufacture of synbiotic drink is made from mangosteen rind. The difference in this research is also on the variables, in the Mawar's study only pH levels were tested while in this study pH levels were tested after fermentation with variations in the time of each sample and the antioxidant activity test after fermentation.

The research conducted by Suharyono et al's (2012) is *L.casei* growth on various fermentation time synbiotic drink of Green Cincau Extract. The similarity of research is in growing *Lactobacillus casei* with variations time of fermentation in the synbiotic drink. The difference with Suharyono et al's (2012) research is in the product of synbiotic drink and the variable of research was to analyse the effect of variations in fermentation time on pH level and antioxidant activity.

CHAPTER II

LITERATURE REVIEW

2.1 Synbiotic Drink

The words ynbiotic is derived from two words namely ‘syn’ means synergy and ‘biotic’ means life. Both these words have meanings that synbiotic is potential synergy between probiotics and prebiotics in food, because the prebiotic substances has a positive effect for intestinal microflora and probiotic bacteria serves as a food substance of probiotics (Yui, 2006).

Consuming synbiotic food or drink can provide a positive impact on the digestive system and provide immunity to the body such as preventing constipation, preventing and reducing colon cancer (Winarti, 2010). Synbiotic is used to regulate the microflora in digestive system because synbiotic is a combination of probiotics and prebiotics. Probiotics are living microorganisms that have a positive impact and prebiotics is the substrate used probiotics for survival. A combination of both can improve the durability of life (Markowiak and Slizewska, 2017).

Prebiotic is defined as a component of undigested food which gives the health effects for the body and stimulates the growth of selectively to certain bacterial activity on colonic (Helmyati et al, 2015). Prebiotic activity has been attached to the various components of food, especially oligosakarida and polysaccharides (including some dietary fiber), but not all undigested carbohydrates and dietary fiber is a prebiotic. (Slavin, 2013). Prebiotic was able to increase the growth of beneficial bacteria (probiotics) that are present in the colon and improve the survival properties, cultivation, as well as the growth of a new probiotic strains are added. The effects of the two are often referred to as the sinergistic effect (Nagpal et al., 2007). Probiotics are a very profitable because microbes can increase bowel mikroflora balance.

Microbial selection especially lactic acid bacteria (LAB) is indispensable to obtain the strains of probiotic strains-superior. Shewale et al. (2014) stated that there are several criteria that must be met by a probiotic, such as: (1) are nonpatogenic and represent the normal microbiota in the gut its host, as well as still active on the condition of the stomach acid and bile salt concentration is high in the intestine, (2) and can grow quickly and are found in high amounts in the intestine, (3) capable of colonized some part of its host, the intestinal tract (4) can produce acid-organic acids efficiently and have antimicrobial properties against pathogenic bacteria, (5) readily produced, are able to grow in large scale production systems, and storage conditions during life.

Probiotics are dietary supplements in the form of microbes beneficial for microbial balance in the intestine. Microbes are used in food or drink fermented dairy-based drink. Fermented milk has been known as a healthy drink. Since 1920, food or drink containing probiotics has been consumed and marketed in Japan. The first bacteria used in the manufacture of fermented milk is *Lactobacillus acidophilus* and *Lactobacillus casei* (Sunaryanto, 2013). Most probiotic bacteria are lactic acid bacteria. Lactic acid bacteria are a group of gram-positive bacteria microaerophilic, does not form spores and can ferment carbohydrates to produce lactic acid and negative catalase. Based on the type of fermentation, lactic acid bacteria are divided into two types i.e.: homofermentative and heterofermentative. Homofermentative bacteria are lactic acid bacteria ferment carbohydrates and produce lactic acid, and heterofermentative bacteria are lactic acid bacteria ferment carbohydrates and produce lactic acid and other compounds such as ethanol, acetic acid and CO2 (Nuryady, 2013). Synbiotic drink which contains probiotics serve to maintain the balance macro ecosystem for people with unhealthy lifestyle, do not pay attention to the cleanliness of food and drink and other factors that can disrupt the balance of microorganisms in the digestive system (Sunaryanto, 2013).

2.2 The Rind of The Mangosteen

Mangosteen (*Garcinia mangostana L.*) is a fruit crop in the area of tropical forest in Southeast Asia. Mangosteen is referred to with a variety of local names in Indonesia such as *manggu* (West Java), *manggis* (Java), *Manggusto* (North Sulawesi) and *Manggih* (West Sumatra) (Prihatman, 2000). Mangosteens are readily found in Indonesia, in which the trees can grow well at an elevation of 0 – 600 m above sea level with an average air temperatures of 20 - 30 º C and pH of soil about 5 – 7, while precipitation is in accordance with the growth of mangosteen trees about 1500 - 300 mm / year, which evenly throughout the year (Mardiana, 2012).

Figure 2. 1 Mangosteen Rind

Source: Rahman, 2018

Results of determination by Indonesian Institute of Sciences (LIPI) Hall, taxonomy mangosteen (*Garcinia mangostana L.*) namely :

Kingdom : Plantae
Division : Spermatophyta
Sub Division : Angiospermae
Class : Dicotyledoneae
Ordo : Malphigiales
Family : Clusiaxceae
Genus : *Garcinia*
Species : *Garcinia mangostana L.*

The content of nutrients in the mangosteen fruit is complete, while the nutrient content of the mangosteen can be seen in the Table 4.2.

Table 2. 1 Mangosteen Fruit Composition Nutritional Value per 100 g

Type of Substance	Total
Energy	34 kcal
Water	87.6%
Protein	0.6 g
Fat	1.0 g
Total Carbohydrates	5.6 g
Fiber	5.1 g
Ash	0.1 g
Calcium	7 mg
Phosphor	13 mg
Iron	1.0 mg
Sodium	7 mg
Potassium	45 mg
Vitamin C	4.2 mg
Vitamin B1	0.03 mg
Vitamin B2	0.03 mg
Niacin	0.3 mg
Xanthones in The Rind	176,76 mg
Xanthones in The Flesh	29 mg

(Source: Lim, 2012)

Mangosteen is famous for the content of pharmacology in addition to having good nutritional content. Almost all parts of the mangosteen tree have the function of each. Portions of the mangosteen consumed is about 32.3% consisting of fruits and seeds, while 29% if only the fruit consumed and the rest of the rind. Mangosteen rind contains water 62.05%, ash 1.01%, 0.71% protein, carbohydrates total 1.17 % and 35.61 % (Permana, 2010).

Figure 2. 2 Mangosteen Rind

Source: Rahman, 2018

One of the chemical contents of the mangosteen rind is xanthone. Xanthone is one of the antioxidant compounds found in the mangosteen fruit. Xanthone has been isolated from all parts of the mangosteen plant (Garcinia mangostana L), especially the rind, fruits, bark, and leaves (Putri, 2015). Xanthone is a cyclic polyphenol ketone because almost all molecular deveratives of xanthones have phenol moieties. Polyphenols are a group of plant chemical characterized by the presence of more than one phenolic group. Polyphenols are responsible for the color of some plants and are considered strong antioxidants which are beneficial for health (HHS, 2011). Antioxidants are substances that the body needs to neutralize free radicals and prevent damage caused by free radicals on normal cells (Halliwell, 2012). Diseases are caused by free radical such as cancer, hypertension, atherosclerosis and nephrotic syndrome (Barhe and Tchouya 2014). Antioxidants can neutralize free radicals by the complete lack of electrons free radicals and inhibit the onset of oxidative stress due to the reaction of free radical formation. Free radical is an atom, group of atoms or molecules that do not have a pair on the outer orbital. Loss of electrons is a cause of free radicals is an unstable molecule. Free radicals will take electrons from molecules or cells in the body of man to be stable. The process of taking electrons from cells causes cell damage (Yuslianti, 2018).

Xanthone derivate includes mengostin, mangostenol A, mangostinon B, trapezifolizanthon, tovophyllin B, α mangostin, β mangostin, garcinon B, mangostanol, epicatechin and gartanin flavonoids. Xanthone is a flavonoid pigments that have a color reaction and chromatography movement similar to the flavonoids but are chemically different. There are fifty types of xanthone isolated from the mangosteen rind include α-, β-, γ-, mangostins, garcinone E, 8, deoxygartanin and gartanin. The isolated xanton has antioxidant activity, anti-tumor, anti-allergic, anti-bacterial and anti-virus. (Widia, 2013). Xanthone which has been isolated from the rind of the mangosteen serves as an anti-cancer, including hepatocellular cancer, breast cancer, and leukemia. α - mangostin has a varied range of bioactivity that is a major compound in the mangosteen because of the activity as an antioxidant, anti-tumor, anti-inflammatory anti-allergic, anti-bacterial and anti-virus (Widia, 2013).

Research by Watanapokasin et al (2010) showed that mangosteen rind is characterized as anti-proliferative and cytotoxic activities both in vitro and in mice. In vitro analysis with a human colorectal adenocarcinoma cell line (COLO 205) showed that mangosteen xanthones do not only inhibit the proliferation of target cells but also induce death associated with apoptosis in tumor cell lines with changes in morphology and oligonucleosomal DNA fragments. Changes in the structure of DNA are caused by free radicals taking electrons from body cells, resulting in mutant cells. If these DNA changes occur for years, cancer will appear. The human body can produce antioxidants, but the amount is often not enough to neutralize incoming free radicals. Therefore humans are advised to consume xanthone (Yatman, 2012).

2.3 Free Radicals

According to Halliwell (2012), free radicals are an atom, group, molecule or compound that has no partner in the outer orbit of the cell. Some of the radical molecules have no pairs are hydrogen, transition metals, and oxygen molecules. Molecules that have no partners will be attracted by the

magnetic field that will change into highly reactive molecules (Yuslianti, 2018). Some diseases are caused by free radicals i.e cancer, cardiovascular disease, diabetic, and other degenerative diseases (Barhe and Tchouya, 2014)

Free radicals found in the human body come from two sources, namely:

- Endogenous sources: autoxidation, enzymatic oxidation, respiratory burst.
- Exogenous sources: drugs, radiation, cigarette smoke. (Winarsih, 2007)

The reaction of free radicals can cause a chain reaction which is capable of damaging the structure of the cells and can lead to various diseases if not terminated One of them is cancer. Antioxidants are necessary to dampen the activity of free radicals. Antioxidants are compounds that can provide electrons to the free radicals, thus stopping the chain reaction of free radical and turning into a stable form

The free radical chain reaction consists of three phases: initiation, propagation, and termination. At the initiation stage the formation of free radicals are highly reactive, it can be caused by the presence of light, oxygen or heat. At the stage of propagation, the radical will form peroxy radicals caused by radicals that react with oxygen. Peroxide radicals will attack the radicals that still have the hydrogen to produce hydrogen peroxide new radicals. Hydrogen peroxide is not stable and would be relegated by carbonyl compounds producing short chain. Fat oxidation reaction will be up to the termination stage if not neutralized by antioxidants (Winarsi, 2007).

2.4 Antioxidant

Antioxidants are compounds that can counteract and reduce free radicals activity by giving electrons to pair with free radicals compounds. Antioxidant will bind and end the process of free radicals chain (Halliwell, 2012). Antioxidants give one electron to complement oxidant compounds

so that the activity of these compounds can be inhibited (Saleh et al, 2012). Termination of free radical chain reactions by antioxidants in the body is important for maintaining a healthy body (Pratiwi and Rustianti, 2015). There are two groups of antioxidants i.e. natural antioxidant which is an antioxidant that obtained from the natural ingredients that have the potential to counteract free radicals and synthetic antioxidant which derived from chemicals (Isfahlan, 2010).

Many natural antioxidants are contained in vegetables and fruits and also found in nuts, seeds, tea, and other food products (Anwar, 2018). In the study of Sen and Chakraborty (2011), showed that vegetable food products generally higher antioxidant content than an animal food product. Classification of antioxidants according to Sen and Chakraborty (2011) divided into several types of antioxidants. Classification of antioxidant types based on their properties include :

a. Enzymatic Antioxidants

Enzymatic antioxidants have a role as the main defense against oxidative stress conditions. These antioxidant enzymes are metalloenzymes whose activities are highly dependent on the presence of metal ions which in turn will prevent the formation of new free radical compounds (Abudhasan et al, 2014).

b. Non-enzymatic Antioxidants

Non-enzymatic antioxidants are secondary antioxidants because they are obtained from outside the body through food intake. Non-enzymatic antioxidants are divided into two parts, are

- Metabolic antioxidants
- Nutritional antioxidant

The classification of antioxidant is also from the source i.e :

a. Endogenous antioxidant
b. Dietary antioxidant
c. Metal binding proteins

(Sen and Chakraborty, 2011)

Antioxidant activity is a compound that can be measured by its ability to give hydrogen atoms to free radical molecules. One method of testing antioxidant activity is the DPPH method with 1,1-diphenyl-2-picrylhydrazyl as a free radical compound centered on organic nitrogen and dark purple. Antioxidants can change radical compounds to become nonradical so they turn clear to yellow. The DPPH test is a test that measures the antioxidant capacity which acts directly with DPPH radicals through monitoring with a UV-Vis spectrophotometer with the absorption of 517 nm (Yu, 2008).

UV-Vis spectrophotometer is a tool used to measure the interaction between electromagnetic radiation and a molecule or atom of a chemical. UV-VIS spectrophotometers can only be done on compounds that have a chromophore group. A chromophore is a functional group that absorbs ultraviolet radiation and appears if it is bound to compounds that are not absorbers (Skoog, 2000). The speed of UV-Vis spectrophotometer analysis is influenced by the absorption spectrum formed. The absorption spectrum is a graph of absorption from absorption to wavelength (Departemen Kesehatan RI, 1995). Antioxidant activity can be calculated with the following formula:

$$\%\ Antioxidant\ Activity = \frac{Abs\ DPPH\ Control - Abs\ DPPH\ Residual}{Abs\ DPPH\ Control}\ x\ 100$$

2.5 Fermentation

Fermentation is a process of chemical changes in the organic substrate through an enzyme produced by microorganisms (Suprihatin, 2010). Starter microbes grown in a substrate are required in the fermentation process. A starter which is microbial population in the amount and physiological conditions were ready inoculated in fermentation media (Prabowo, 2011). There are two ways of fermentation namely: spontaneous and not spontaneous. Spontaneous fermentation is fermentation that is not added microorganisms in the form of a starter or yeast in the manufacturing process. Non-spontaneous fermentation is fermentation or yeast starter which is added during the manufacturing process (Suprihatin, 2010). Several factors

affect the fermentation: temperature, pH of the initial fermentation, the inoculum, substrate and nutrient content of the medium (Hidayat, 2006).

2.5.1 *Lactobacillus casei*

Lactobacillus casei is a bacterium that has a stem-shaped morphology, in a single or chain colony, gram-positive, negative catalase, does not form endospores and capsules, and does not havr flagella. Based on its growth temperature, these bacteria include mesophyll bacteria that can live at the temperature of 15-41ºC and pH 3.5 or more, while optimum conditions for growth is at 37 ºC and pH 6.8 (Najgebauer et al., 2011). *Lactobacillus casei* is homofermentative, i.e. can break down glucose into lactic acid (approximately 90%) and produces citric acid, malic, succinic, acetic, acetaldehyde, diacetyl and acetoin that instrumental in the formation of flavour (Su-Yeon, 2008).

Lactobacillus casei culture makes the acidity of milk low. Step work of *Lactobacillus casei* culture begins with adding the culture into the milk that has gone through a process of heating at 90 ºC for 15-30 minutes and then cooled down to 43 ºC (Dwidjoseputro, 2005). Fermentation starts when the activity of the *Lactobacillus casei* converts lactose to lactic acid and decrease the acidity of milk to 3.5 or more which results in higher lactic acid levels. The tendency for chemical reactions to occur during fermentation which can be detrimental to the final product begins to be inhibited to create a distinctive taste of fermented products (Dwidjoseputro, 2005).

Lactic acid bacteria is one kind of prebiotics which is known to have an anti-cancer effect. Research conducted by Daniluk (2012) showed that *Lactobacillus acidophilus* can reduce 3 enzymes i.e. β-glucuronidase, azoreductase, and nitroreductase which has an important role for the formation of carcinogenic compounds which can catalyze procarcinogens into carcinogenic compounds in the colon. Jacouton et al (2018) showed that *Lactobacillus casei* injected in intraperitoneal, intravenous and subcutaneous mice showed antitumor

activity and increased immune response through increased macrophage function, natural killer cell activity, and T lymphocyte cells.

2.5.2. The Degree of Acidity (pH)

The degree of acidity (pH) is one of the important indicators in the process of preserving food, in which the importance of pH caused by the pH related premises microbial survival. Food will be more durable if the pH level is getting lower due to the fact that panthogen bacteria cannot survive at low pH (Lund et al, 2014). During the fermentation process, pH buffer is often added to the substrate to reduce or slow down large pH changes and also had a role as a source of nutrients. When pH conditions determine the growth of microorganisms, lactic acid bacteria will grow at pH 3.0-6.0 (Afrianti, 2014). The pH value of the test results showed the concentration of H + ions in solution. The pH value will be lower if the concentration of H + ions in a solution is high. Measurement of the degree of acidity (pH) can only be performed on a solution that produces lactic acid (James et al, 2018).

2.5.3. Time of Fermentation

Long fermentation time had an influence on the total lactic acid bacteria synbiotic drink because total lactic acid bacteria will affect the pH value in the synbiotic drink (Hidayat, 2013). The growth of lactic acid bacteria is one of the characteristics or properties of the microorganisms in fermentation. According to Andriani 2010, the growth of lactic acid bacteria is divided into 5 phases i.e.: adaptation phase, initial growth phase, slow growth phase, stationary phase, and death phase.

In a study conducted by Suharyono (2012), at 0-8 hour fermentation, the increase on the number of bacteria is to slow because the bacteria were still in a phase of adaptation so that the bacterial growth was not optimal. On the 8-16 hour fermentation, the bacteria have been in logarithmic phase growth phase or initial growth phase

where lactic acid bacteria divide quickly and constant logarithmic curve follow. At 24 hour fermentation, bacteria growth began to decline but remains relatively constant and the value of its descent is not too significant, which is the value indicates that the lactic acid bacteria is on permanent growth phase. In the stationary phase, it will occur if all cells stop diving or living cells and dead cells reach a balance (Andriani, 2010). Since death phase occurs after 40-48 hours of fermentation, this phase is characterized by a decrease in the number of population of lactic acid bacteria caused by the depletion of nutrients in the media. The number of cells that died will cause a decrease in the number of microbial populations (Suharyono, 2012).

2.6 Islamic Views on the Utilization of Mangosteen Rind as a Functional Food

Indonesia is a country which is rich in plants. A diversity of the plants has been widely used by Indonesians as a food ingredient to prevent or treat a variety of diseases. Allah SWT created all creatures with the function of each. The science needs to know the functions of plants that have been created so that its benefits can be taken.

Allah says in Q.S Thaahaa/20: 53 :

الَّذِي جَعَلَ لَكُمُ الْأَرْضَ مَهْدًا وَسَلَكَ لَكُمْ فِيهَا سُبُلًا وَأَنْزَلَ مِنَ السَّمَاءِ مَاءً فَأَخْرَجْنَا بِهِ أَزْوَاجًا مِنْ نَبَاتٍ شَتَّىٰ (٣٥)

"He Who has made for you the earth like a carpet spread out; has enabled you to go about therein by roads (and channels), and has sent down water from the sky. With it have We produced diverse pairs of plants each separate from the others."

The verse above explains that Allah has created man and the existence of humans on earth and the ways that humans have taken to get their goals are guidance from Allah. Allah created humans and animals to utilize fruits and plants for the continuation of their lives so that Allah sends down rain

which is part of Allah's guidance for plants to grow and develop (Shihab, 2009)

Mangosteen plant is a type of tropical plant that depends on rainfall that can be utilized by humans as a functional food.

Allah says in Q. S, An- Nahl : 10-11

هُوَ الَّذِي أَنزلَ مِنَ السَّمَاءِ مَاءً لَكُمْ مِنْهُ شَرَابٌ وَمِنْهُ شَجَرٌ فِيهِ تُسِيمُونَ (١٠) يُنْبِتُ لَكُمْ بِهِ الزَّرْعَ وَالزَّيْتُونَ وَالنَّخِيلَ وَالأعْنَابَ وَمِنْ كُلِّ الثَّمَرَاتِ إِنَّ فِي ذَلِكَ لآيَةً لِقَوْمٍ يَتَفَكَّرُونَ (١١)

> *"He is Who sendeth down water from the sky, whence ye have a drink, and whence are trees on which ye send your beasts to pasture (10) Therewith He causeth crops to grow for you, and the olive and the date-palm and grapes and all kinds of fruit. Lo! herein is indeed a portent for people who reflect (11)"*

The verse is the detail that Allah is the One. This paragraph describes the plant as a food both human and animal need. Plants can only grow because they are grown by Allah SWT, so this verse reminds people to be grateful for God›s blessings and make good use of them (Shihab, 2009).

Based on the verse above it can be seen that God created various kinds of plants to be used by humans. Mangosteen rind is one part taken from the mangosteen fruit which is used in this study so that it can be known its benefits as functional food ingredients and as a source of antioxidants (Shihab, 2009).

As the hadith narrated by Abu Hurairah R.A, Prophet Muhammad SAW said :

حَدَّثَنَا مُحَمَّدُ بْنُ الْمُثَنَّى حَدَّثَنَا أَبُو أَحْمَدَ الزُّبَيْرِيُّ حَدَّثَنَا عُمَرُ بْنُ سَعِيدِ بْنِ أَبِي حُسَيْنٍ قَالَ حَدَّثَنِي عَطَاءُ بْنُ أَبِي رَبَاحٍ عَنْ أَبِي هُرَيْرَةَ رَضِيَ اللَّهُ عَنْهُ عَنْ النَّبِيِّ صَلَّى اللَّهُ عَلَيْهِ وَسَلَّمَ قَالَ مَا أَنْزَلَ اللَّهُ دَاءً إِلَّا أَنْزَلَ لَهُ شِفَاءً

> *"Having told us [Muhammad bin Al Mutsanna] had told us [Abu Ahmad Az Zubairi] had told us [‹Umar bin Sa›id bin Abu Husayn] he said; has told me [‹Atha` bin Abu Rabah] from [Abu Hurairah] Radli Allahu› anhu from the Prophet Sallallaahu ‹alaihi wasallam he said: «Allah will not reduce disease but provide the medicine too.»* (H.R. Bukhari No. 5246)

The hadith explains that all diseases given by Allah must have a deterrent effect or it will be healed by Allah›s permission. Mangosteen rind can be disease prevention because it has high antioxidants. Meanwhile, cancer is a disease that can be prevented by consuming foods or drink that contain antioxidants.

2.7 Theoretical framework

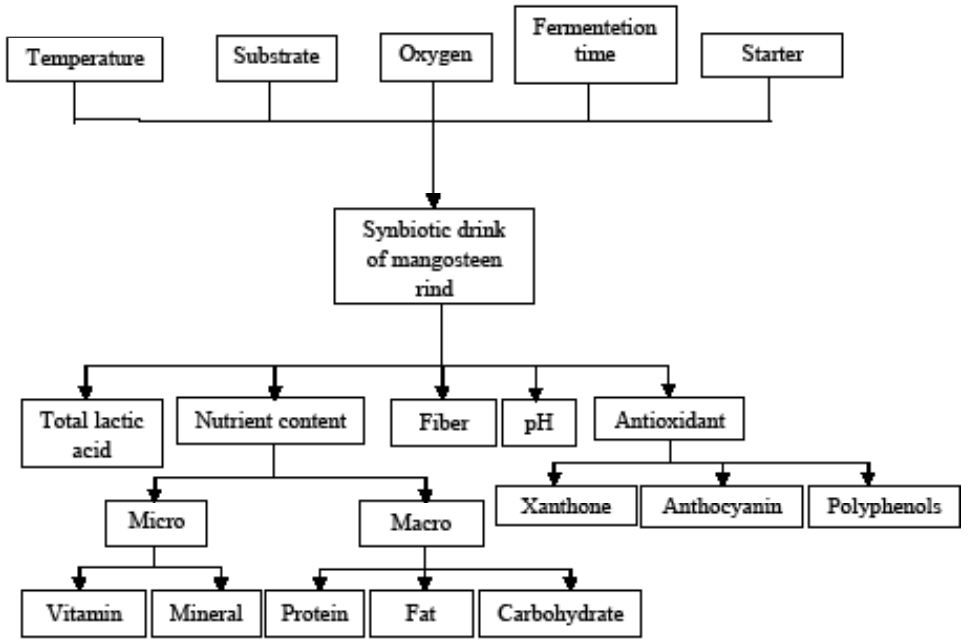

Figure 2. 3 Theoretical Framework

Source: Iswari et al. (2005), Desrosier (2008), Kemenkes, 2010

2.8 Conceptual Framework

From the theoretical framework above, the researcher wanted to analyze the effect of time variation on fermentation oh pH level and antioxidant on a synbiotic drink of mangosteen rind.

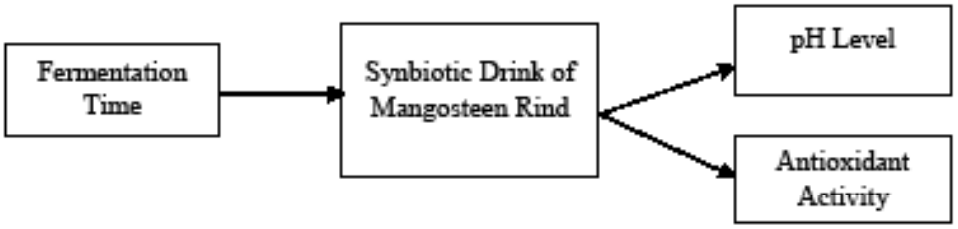

Figure 2. 4 Conceptual Framework

2.9 Research Hypothesis

2.9.1 Major Hypothesis

There is an effect of the variation of fermentation time on the pH level and antioxidant activity of synbiotic drink of mangosteen rind.

2.9.2 Minor Hypothesis

a. There is an effect of fermentation time on the pH level of the synbiotic drink of mangosteen rind.
b. There is an effect of fermentation time on the antioxidant activity of synbiotic drink of mangosteen rind.

CHAPTER III

RESEARCH METHODS

3.1 Research Design

The research conducted was experimental research to determine the effect of differences in fermentation time. In this study, several steps are carried out, i.e. the preparation stage of the sample, the stage of fermentation, the pH test, and the test of antioxidant activity. This study used a Randomized Block Design (RBD) with a long fermentation factor of 12 hours, 24 hours, 36 hours and 48 hours (Suharyono, 2012).

3.2 Time and Place of Research

The research was conducted in December 2018 to February 2019. Manufacture of synbiotic drink and pH levels test was carried out in the Laboratory of PAU Gadjah Mada University. Antioxidant activity test was carried out in Chem-mix Laboratory

3.3 Tools and Ingredients

3.3.1 Tools

The tools used in this study are: analytical scales, test tubes, test tube racks, beaker, erlenmeyer flask, stirring rods/spatulas, micropipette, microtip, aluminum foil, cuvette, blenders, static, freezer, evaporator flask.

The tools used for the analysis include :

- Antioxidant activity analysis: UV-Vis spectrophotometer, 200 rpm speed centrifuge, 25 ml Erlenmeyer, propylene tube, vortex mixer, 1 ml pipette a volume, and micropipette
- pH analysis: pH meter

3.3.2 Ingredients

The main ingredients used in this study ware the inside of

the mangosteen rind (*Garcinia mangostana L.*) obtained from the traditional market of Sewon, skim milk, *Lactobacillus casei* bacterial cultures obtained from PAU UGM, sugar, and aquades.

The materials used for the analysis include :

- Analysis of antioxidants: 2,2-diphenyl-1-picryhlydrazyl (DPPH) and ethanol
- pH analysis : the sample solution

3.4 Operational Definition

Tabel 3. 1 Operational Definition

Variable	Operational Definition	Scale	Indicator
Mangosteen Rind	The inside rind of the mangosteen fruit selected from fresh mangosteen fruit are obtained from traditional market in Sewon	Ratio	Rind from fresh fruit.
Synbiotic Drink of Mangosteen Rind	Synbiotic drink of mangosteen rind is a drink from the mangosteen rind with the addition of skim milk, sugar, and fermented using *Lactobacillus casei* bacteria	Ratio	pH 4,0-4,5, sour taste (SNI, 2009)
Fermentation Time	The time required in the process of changing the chemistry of organic substrate through the enzyme activity produced by microorganism	Ratio	12 hours, 24 hours. 36 hours and 48 hours (Suharyono, 2012)

pH Level	The pH value shows the acidity of the synbiotic drink of mangosteen rind with a variation of fermentation time	Ratio	4,0-4,5 (SNI, 2009)
Antioxidant Activity	The ability of synbiotic drink of mangosteen rind to capture DPPH free radicals	Ratio	There was aChange the color of the solution from purple to yellow (Akar, 2017).
DPPH Method	A nitrogen radical compound is used to measure the capacity of an antioxidant	Ratio	Wavelength of 517 nm (Prakash, 2001).
Inhibition concetration 50%	The value of synbiotic drink of mangosteen rind with fermentation time can produce the highest 50% DPPH radical capture	Ratio	Very strong : <50 ppm Strong : 50-100 ppm Moderate : 100-150 ppm Weak: 150-200 ppm (Blois, 2005).

3.5 Research Procedure

3.5.1 Making Synbiotic Drink of Mangosteen Rind

a. Making Mangosteen Rind Juice

Making mangosteen rind juice is done by using the methods of Dewi and Ayu (2014) and modified as follows: the mangosteen rind that has been separated from the fruit is cleaned and cut into smaller pieces. Next was destruction by adding water twice as much as mangosteen rind. Mangosteen rind has been destroyed then filtered using a filter cloth and subsequently precipitated, the

precipitated ones are separated from the filtrate and then the filtrate is pasteurized at 90ºC for 15 minutes.

b. Starter Preparation Process

Preparation of the starter is to modify the Suharyono's (2004) method. *Lactobacillus casei* which used was transferred from the stock culture into test tubes contains a sterile Broth media, then incubated for 24 hours on 37ºC. *Lactobacillus casei* culture 4% inoculated into media sterile skim milk contains 5% (0.5 g skim milk in 10 ml aquades). Sterilized at 121 ºC for 15 minutes, then incubated for 48 hours at 37 ºC. The result culture is the main culture. The main culture inoculated to media containing sterile skim milk 4% (v/v) (0.4 ml the main culture added into 9.6 ml skim milk medium) then incubated for 48 hours at 37ºC. The resulting is the secondary culture. The secondary culture inoculated to media containing sterile skim milk 4% (v/v) (0.4 ml the main culture added into 9.6 ml skim milk medium) added with sucrose 3% (w/v) then incubated for 48 hours at 37 ºC, the resulting culture is addressed by work culture. In the process of making a synbiotic drink used 4% (v/v) work culture as a starter.

c. Making Synbiotic Drink of Mangosteen Rind

Making a synbiotic drink is done by Suharyono et al, (2017). 2% (w/v) of skim milk, 2% of sucrose, and added mangosteen rind juice with a concentration of 15% (w/v), then added aquades until the volume becomes 115 ml then the mixture is stirred until blended using a spatula glass for 30 seconds, the next step was pasteurized 76 ºC for 15 minutes, then cooled into 37 ºC. The work of *Lactobacillus casei* was incubated in an incubator at 37 ºC for 12 hours, 24 hours, 36 hours and 48 hours.

3.5.2 pH Analysis

This study used pH test used by Sudarmadji's (2010) method. The samples which were homogenized (fermentation medium) are

taken approximately 30 ml and placed into 50 ml glass beaker. The pH meter is calibrated before use by using buffer pH 7 and 4. Then the pH meter is turned on and allowed 15-30 minutes to be stabilized. PH meter electrode was rinsed with distilled water and dried with a paper towel. Then the electrode are dipped into the sample and the pH meter is adjusted, the electrodes are allowed to be immersed in the solution until a stable reading is obtained.

3.5.3 Testing Antioxidant Activity with DPPH

a. Preparation of DPPH Solution

Antioxidant activity are determined by methods spectrophotometric with 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Farhan, 2012). DPPH solution was used as a receptor on a sample of 15 mg DPPH dissolved in 150 ml methanol and homogenized (DPPH solution 1). The DPPH solution then was determined its absorption spectrum using a UV-Vis spectrophotometer with wavelength 0f 517 nm. The blank solution used was 1 ml of methanol was added 2 ml DPPH 1 and 1 ml of methanol was added and homogenized and incubated at 37 ºC for 30 minutes and measured at a wavelength of 517 nm to obtain blank absorbance (Rahim, 2012).

b. Preparation of Test Solution

Synbiotic drink of mangosteen rind is considered about 10 mg and then diluted with 10 ml of methanol so that the concentration of the solution was 1000 ppm. Making a series solution of 50, 100, 150, 200, 250 ppm started from mother liquor 0,5; 1,0; 1,5; 2,0; 2,5 ml and put in a vial bottle an then add methanol until the volume reaches 10 ml and incubate (Rahim, 2012).

c. Testing of Antioxidant Activity In Synbiotic Drink of Mangosteen Rind

50, 100, 150, 200, 250 ppm series solution are pipetted 1 ml then added 2 ml DPPH 1 then added 1 ml of methanol. The solution was shaken until it becomes homogeneous and incubated at 37ºC for 30 minutes and measured at a wavelength of 517 nm to obtain DPPH absorbance. The results obtained absorbance, was added to the formula to find % inhibition of each solution

Antioxidant activity is calculated by the formula:

$$\%\ Antioxidant\ Activity = \frac{Abs\ DPPH\ Control - Abs\ DPPH\ Residual}{Abs\ DPPH\ Control}$$

x 100

Information :

Abs DPPH Control = Absorbance before reacted with a sample DPPH

Abs rest of DPPH = Absorbance after reacted with a sample DPPH

IC_{50} values which are calculated as a percentage inhibition value of each concentration was found. The regression equation IC_{50} values are as follows:

Y = a + bx

Information :

Y : % Inhibition (i.e. 50)

X : The value of IC_{50}

3.6 Data Analysis Method

In this experiment, direct experiments were conducted on the divided samples of the synbiotic drink of mangosteen rind based on the variation of fermentation made by the researcher. The results obtained using the formula included to obtain % antioxidant activity. The analytical method used in this research is the method of analysis of variance (ANOVA) through was used to obtain a conclusion about the effect of treatment. Determination of rejection of hypothesis (H_0) is done after conclusions are obtained, i.e.:

1. H_0 is accepted if F count <F table, H_1 is rejected
2. H_0 is rejected if F count> F table, H_1 is accepted

The information above shows that H_1 is accepted if there are significant differences among the averages of each treatment. The analysis was carried out if there were significant differences in each treatment, namely by the Tukey test to find out the group of samples that had a significant difference at the significance level $\alpha = 0.05$. Data inhibition and concentration of the solution used to search for IC_{50} values. IC_{50} is a concentration level parameter that can inhibit cancer cell growth by 50% (Arifianti, 2014). The lower is IC_{50} value the higher is the anticancer activity (Winarno, 2011).

3.7 Discussion Systematics

The experimental research carried out began with making a synbiotic drink of mangosteen rind. Synbiotic drink of mangosteen rind which is fermented with various fermentation times, i.e. 12 hours, 24 hours, 36 hours and 48 hours were then tested for antioxidant activity using the DPPH method and measured the degree of acidity using a pH meter. The results of the antioxidant activity obtained were calculated as % inhibitors and then counted IC_{50} using a linear regression equation to determine the antioxidant strength of the synbiotic drink of mangosteen rind. The results obtained

were compared for each sample with various fermentation times so that the antioxidant activity and the best pH level were known in the synbiotic drink samples of mangosteen rind with variations in time of fermentation.

3.8 Plan of Research Time

Tabel 3. 2 Plan of Research Time

No	Activities	Schedule of Activities					
		Nov	Dec	Jan	Feb	Mar	Apr
1	Preparation of thesis proposals	■					
2	Proposal seminar		■				
3	Material purchase		■				
4	Research		■	■			
5	Reporting				■	■	
6	Examination					■	
7	Revision					■	■

CHAPTER 4

RESULT AND DISCUSSION

4.1 Effect of Fermentation Time on pH Value of Synbiotic Drink of Mangosteen Rind

Analysis of pH value or acidity in the synbiotic drink of mangosteen rind was carried out after fermentation in which pH analysis aimed to determine alteration in pH value of synbiotic drink of mangosteen rind during the fermentation process. Measurement of pH value is donedirectly using a pH meter. The result of the pH values in this study can be seen in Figure 4.1 below.

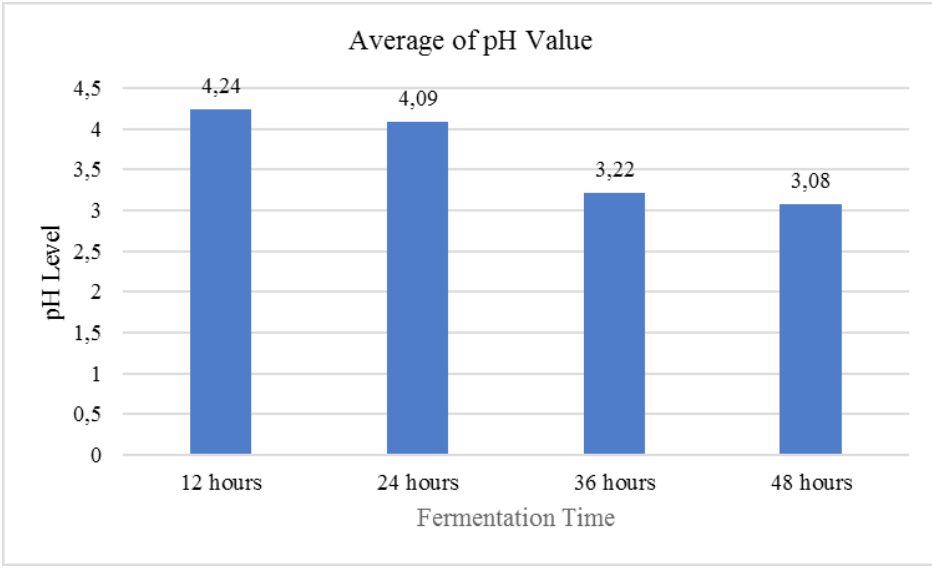

Figure 4. 1 Graph of Average Value of pH Synbiotic Drink

Figure 4.1 shows that the average pH value in the synbiotic drink of mangosteen rind has declined with the length of fermentation time. A decrease in pH value is related to total acid in the synbiotic drink which is the result of microbes. Microbes growth at the time of fermentation used carbohydrates in sugar for metabolic processes and the length of

fermentation time had an influence on the higher level of total acid. The increase of total acid in the synbiotic drink of mangosteen rind is measured by a decrease in pH. According to Hardayi et al (2013), decrease in pH is caused by the formation of lactic acid from lactic acid bacteria metabolism.

The average pH value of the product in this study ranged from 3,08 to 4.24 with 12, 24, 36, and 48 hours fermentation. Standar Nasional Indonesia (01-2982-1992) stipulates that fermented milk drink is good if it has a pH value approximately 4.0 to 4.5. In this study, samples with a 12 hour and 24 hour fermentation period met SNI with a pH of 4.24 and 4.09.

Based on the results of the normality and homogeneity test, it is obtained that the data is normal distribution and homogeneous data variables. Based on the results of One Way ANOVA statistical analysis with confidence interval 5% concluded that Fcount > Ftable which means that fermentation time is affected on the pH value of synbiotic drink of mangosteen rind. The results of the one way ANOVA statistical analysis stated that Ho was rejected, further test was carried out with a Tukey test. The results of further tests on the effect of fermentation time on the pH value of synbiotic drink in this study canbe seen in Table 4.1 below.

Table 4. 1 Tukey Test on pH Value Synbiotic Drink

Fermentation Time	Mean	±	SD	p-value
12 hours	4.24	±	0.012[a]	0.000
24 hours	4.09	±	0.010[b]	
36 hours	3.22	±	0.200[c]	
48 hours	3.08	±	0.173[d]	

Different letters indicate significant differens between groups

Based on Table 4.1, it can be seen that the effect of fermentation time on pH values showed significant differences. Significant differences in pH values occur because synbiotic drink has been divided into several groups of different fermentation time. The longer fermentation time has caused microbes to break down a lot of sucrose into glucose and fructose

through invertase enzymes (Ramesh et al, 2016). The research conducted by Kumala (2011) shows that the carbon source and energy source of lactic acid bacteria for growth and production lactic acid is fructose.

One factor which causes a decrease in pH level on synbiotic drink is the duration of fermentation. During the fermentation time of 12 to 48 hours, the amount of lactic acid formed from *L.casei* has been increased. Increasing the amount of lactic acid in fermentation is measured by pH level starting from the 12 hours fermentation time with pH level 4.24. Lactic acid produces a large amount of H^+ at pH 4 because it is susceptible to dissociation. pH measurements with a pH meter indicates that the greater H^+ ion are measured, the lower the pH value (Sayuti et al, 2013).

The decrease in pH value in Figure 4.1 is also caused by the base ingredients used in making synbiotic drink of mangosteen rind. Mangosteen rind, skim milk, and sugar-containing complete nutrients such as proteins, carbohydrates and fats are good media and nutrients for the growth of microorganisms, mainly lactic acid bacteria (Muchtadi, 2010). Synbiotic drink of mangosteen rind ferments with the presence of bacteria *L.casei* converts sucrose to alcohol and ends up as acid (Ao et al, 2012).

Decomposition of sucrose by lactic acid bacteria produce carbon and energy for bacterial activity to produce lactic acid bacteria (Lahtinen et al, 2011). *L.casei* removed lactic acid from cells that accumulate in the fermented products. A decrease pH in a synbiotic drink of mangosteen rind caused by acid is formed by lactic acid as the main product of lactic acid bacteria metabolism (Papagianni, 2012).

The results of one way ANOVA (Table 4.1) showed that the storage time on each fermentation time had a significant effect on pH value of synbiotic drink. The lowest pH level of 3.08 was seen in the sample at 48 hours fermentation time. Meanwhile, the pH level at 36 hours and 48 hours fermentation show a relatively lower number and significant difference. It can be assumed that treatments with 36 and 48 hours fermentation have pH levels did not meet the standard value.

Determination of fermentation time affected the organoleptic properties of lactate fermented drink product. The duration of fermentation affected the characteristics of the fermented drink due to the difference in the total acid produced by LAB affected the decreasing pH of the product and also the flavor produced. The long fermentation time which produces synbiotic drinks with a low pH due to the continuation of lactic acid production by lactic acid bacteria causes increased levels of total acid and the growth of lactic acid bacteria (Suharyono, 2012).

Low pH causes the taste of synbiotic drink to become too sour which might reduce the consumer. The pH level in this study is about 4.24 to 3.08. Low pH level of synbiotic drink in 36 and 48 hours of fermentation may be influenced by two factors: 1) the effect of the pH on mangosteen rind extract or, 2) performance of the antioxidants contained in the mangosteen rind extract. The first possibility is the pH value of mangosteen rind extract of 5.28 (Damiyanti et al, 2014). As for the second possibility, it is based on a research reported by Zeuthen and Sorensen (2003) the symbiosis between lactic acid bacteria and antioxidant substances.

From the results obtained, samples for 12 and 24 hours fermentation showed significant differences but it can be inferred that the product which is still eligible for consumption due to the acidity of the product is still in the normal range. These results also indicate that mangosteen rind can retain the pH of synbiotic drinks during the storage period. Low pH in the product plays a role in suppressing the growth of other undesirable microorganisms. Mattila-Sandholm and Saarela (2000) suggested that low pH in fermented milk, lactic acid and flavor compounds inhibited the growth of most of the panthogen bacteria in milk to produce safe and healthy products. *Lactobacillus* has a role in controlling intestinal microorganisms and reduce the formation of toxic products in the digestive tract.

Chemical processes in the formation of synbiotic drinks are divided into two stages, including the stages of reforming lactose into lactic acid, and the reaction of lactic acid with calcium contained in casein (Tamine and

Robinson, 2000). pH level which is decreased is in this study decreased in line with the growth of lactic acid bacteria, so the addition of mangosteen rind extract containing antioxidants does not inhibit the fermentation process at a certain degree. pH level in normal range after storage process indicates a capable antioxidant performance which maintains the consistency of the solution from the effect of oxidation.

An indication the influence of antioxidants in the fermentation process of making synbiotic drink may be caused by the fermentation process as a reduction reaction between lactic acid and calcium bound to the casein proteins (Mattila-Sandholm and Saarela, 2000). Zeuthen and Sorensen (2003) explains that pH affected the energy metabolism of pathogenic microbes by inhibiting transport or water exchange on cell membranes, and the stability of microbial cell macromolecular. Some microorganisms have the natural ability to survive from a wide pH tolerance due to having a passive pH homeostasis in the membranes. Microorganisms prevents the external proton to enter cells by increasing the capacity of cytoplacmic buffer or glutamate synthesis or citrate in the cytoplasm. Microorganisms with passive pH homeostasis have a low membrane permeability on protons and ions.

Lactobacillus casei is a microorganism with an active pH homeostatic membrane (Broadbent et al, 2010). Zeuthen and Sorensen (2003) stated that bacteria that have active pH homeostatic are bacteria with cytoplasmic pH which are formed in the metabolic activity by the active transport of protons and ions. Cytoplasmic pH of these bacteria increase and change depending on environmental conditions so the changes in certain pH metabolic activity will be inhibited. Antioxidants indirectly prevent the entry of protons actively into cell membranes thus stability of pH in the bacterial cytoplasm can be relatively maintained (Tamine and Robinson, 2000).

Research conducted by Suhayono (2012) showed the product after fermentation for 32 hours or over which has a lower pH level is not significant due to the ability of microbes to break down organic compounds

has decreased. When lactic acid bacteria have been entered the death phase, thus decrease in pH is not significant. A decrease in pH still which occurs as a result of accumulation total acid, not as an essential acid (lactic acid) but a short chain weak acid such as acetic acid, propionate, and butyrate which are the result of lactic acid hydrolysis.

4.2 Effect of Fermentation Time on Antioxidant Activity of Synbiotic Drink of Mangosteen Rind

Research conducted on the test of antioxidant activity on the synbiotic drink of mangosteen rind was carried out using the DPPH method. After samples mixed with DPPH, the sample was incubated for 30 minutes. Luo (2011) explains that the optimal reaction time between DPPH and antioxidants is 30-40 minutes. From the results of research conducted on the synbiotic drink of mangosteen rind samples the following results were obtained in Table 4.2 below.

Tabel 4.2 Tukey Test on Antioxidant Activity Synbiotic Drink

Fermentation Time	Mean	±	SD	p-value
12 hours	160.292	±	1.627[a]	0.000
24 hours	146.409	±	1.756[b]	
36 hours	123.485	±	6.126[c]	
48 hours	92.200	±	5.563[d]	

Different letters indicate significant differens betwen groups

Antioxidant activity testing was carried out by making DPPH stock solution by weighing DPPH powder as much as 15 mg and dissolved with 100 ml methanol and make stock solution from synbiotic drink of mangosteen rind with various concentrations of 50 ppm, 100 ppm, 150 ppm, 200 ppm, 250 ppm. Then the solution is incubated in the incubator for 30 minutes at 37ºC to react with DPPH solution. Absorbance measurements were carried out by UV-Vis spectrophotometry at 517 nm wavelength with three-times replication for more accurate results.

Based on the results of Table 4.2, from the average absorbance obtained then the calculation of % inhibition is done to get the standard curve. After the standard curve obtained, the absorbance results are calculated by linear equations of lines to get the IC_{50} value. From the data shown in the synbiotic drink of mangosteen rind b value means that for every x (sample concentration) increases, then y (% inhibition) increased by b value. Antioxidant activity was measured based on the decay of purple when DPPH solution was mixed with antiradical material, the decay of color as a result from hydrogen reaction of antioxidant against DPPH (Akar et al, 2017).

According to Sudjana (2003) if the linear regression equation value of b is positive, it is shown that inhibition of antioxidant value curve increases. The coefficient b is the linear regression direction coefficient and shows the changes in the average variable y for each change in variable x. Based on linear equations obtained the IC_{50} value on synbiotic drink of mangosteen rind at fermentation time of 12 hours was 160.292 ppm, 24 hours was 146.409 ppm, 36 hours was 123.485 ppm, and 48 hours was 92.200 ppm. IC_{50} is the number to indicate the concentration of samples in inhibit the oxidation process up to 50%. The smaller IC_{50} value means the higher antioxidant activity.

According to Blois (2005) a compound has a very strong antioxidant activity if the IC_{50} value is less than 50 ppm, strong (50 ppm-100 ppm), medium (100 ppm-150 ppm), and weak (150 ppm-200 ppm). Based on the results obtained, synbiotic drink of mangosteen rind showed weak antioxidant activity at 12 hours fermentation time. 48 hours fermented products produce stronger antioxidant activity.

Table 4.2 indicates that the level of antioxidant activity has strengthened in line with the length of fermentation time with a significant difference. The results in this study showed that antioxidant activity in early fermentation tends to be weakened but strengthened until the end of fermentation time. A weak antioxidant activity is possible because

anthocyanin contained in mangosteen rind decomposes. In the process of making mangosteen rind extract, pasteurization step has been done at a temperature 90^{o}C for 15 minutes. According to Puspita (2010), high temperatures led to the decompose anthocyanin structure and continued heating led to structural degradation. The loss of anthocyanin levels due to heating is irreversible because colorless chalcons cannot return to being red flavillium cations. Research conducted by Puspita (2010) showed that the stability of red rosella anthocyanin on temperature of 70^{o}C during 30 minutes showed a decrease in the content of 47.45%. Anthocyanin is one of the antioxidant compounds in mangosteen rind (Chaovanalikit, 2012). Unzilarimbi (2012) states that the heat transfer causes antioxidant damage. Anthocyanin is highly sensitive to the thermal processes in which the colors that have been disappeared and turned brown due to the degraded and polymerized pigments.

Based on Table 4.2 it also can be inferred that longer fermentation time can increase antioxidant activity. Increased antioxidant activity was associated with a decrease in pH level due to the length of fermentation time. In this study, the rate of decrease in pH is directly proportional to the rate of increase in antioxidant activity. Increased antioxidant activity is probably caused by organic acids as a result of lactic acid bacteria (*L.casei*) metabolism during fermentation. Organic acids have been given H^{+} ions to the radical so the primary antioksidant activity increased. High capture of free radicals on synbiotic drink of mangosteen rind is caused by the presence of alpha-mangostin compounds, anthocyanin compounds and total phenolic which are the main scavenger compounds among other bioactive components (Chaovanalikit, 2012).

Increased antioxidant activity may be caused by the formation of lactic acid. Based on research conducted by Prestes et al (2013) that lactic acid in yogurt contains α hydroxy acids (AHA) as an antioxidant and often used for the manufacture of cosmetics. Antioxidant activity was influenced by lactic acid (CH3-CHOH-COOH) produced by probiotic bacteria as a

hydrogen atom donor for molecules or atoms that have unpaired electrons on outer orbit (free radicals). The color decay of DPPH solution in antioxidant activity test caused by the donation of hydrogen atoms in unpaired electrons from group N on DPPH structure. The stronger antioxidant activity contained in the product it can reduce the intensity of the color purple which are increasingly significant.

The activity of probiotic bacteria which has produced the compound acts as an antioxidant and causes an increase in antioxidant activity. Antioxidant activity contained in the fermented milk is a natural antioxidant derived from probiotic bacteria during the fermentation process. An antioxidant is a secondary metabolite of bacterial metabolism. Probiotic bacteria initiate the formation of secondary metabolites when entering the stationary phase (Andriani, 2010). Tayo and Akpeji (2016) stated that during storage, the probiotic LAB candidate grew and had significant increased vitamin C content.

4.3 Integration of Science and Al-Quran for Synbiotic Drink of Mangosteen Rind

Fermented drinks such as synbiotic drinks are drinks that have more benefits than a drinks without fermentation (Hidayat, 2006). In fermented drinks, the acids formed can extend the shelf life to prevent the growth of decompose microorganisms and panthogen microorganisms, thus increasing product safety. Allah says in ‘Abasa/80: 24

فَلْيَنظُرِ ٱلْإِنسَٰنُ إِلَىٰ طَعَامِهِۦٓ (٢٤)

“Let man consider his food”

The word “ينظر”, based on the tafsir of Sayyid Qutub (2007), can be interpreted to see or reflect or think that food is the closest to humans because it is the primary need of every human being. By paying attention to the affairs that occur repeatedly, humans will understand the amazing story of the power of God so that the food makes God more fearful.

Based on this interpretation, it can be seen that one process to pay attention to food is to look at the content of foods or drinks that are good for consumption, such as synbiotic drinks. This study is evidence that the effect of fermentation time on the synbiotic drink of mangosteen rind can increase antioxidant activity. The levels of antioxidant activity in this study ranged from 160,293 – 92,265 ppm. Therefore, people should know the content or nutrition of food and drinks consumed. Allah says in An-Nahl: 114 which is the criteria of food that can be consumed by humans

فَكُلُوا مِمَّا رَزَقَكُمُ اللَّهُ حَلَالًا طَيِّبًا وَاشْكُرُوا نِعْمَتَ اللَّهِ إِنْ كُنْتُمْ إِيَّاهُ تَعْبُدُونَ (١٤٤)

"Then eat of what Allah has provided for you (which is) lawful and good. And be grateful for the favor of Allah, if it is (indeed) Him that you worship."

Based on the tafsir of Ibn Kathir (2007), the above verse explains that Allah SWT instructs humans to consume foods that are good for the body, because food and drink are needs that must be fulfilled every day by humans. The word "lawful" and "thayyib" is an argument to consume foods that are healthy, proportioned, secure, and lawful. Synbiotic drink of mangosteen rind used *Lactobacillus casei* in fermentation process. Lactic acid as a result of the metabolism of lactic acid bacteria has many functions in the health of digestive system in the intestine (Saez-lara et al, 2015).

عَنِ ابْنِ عَبَّاسٍ قَالَ كَانَ رَسُولُ اللَّهِ صَلَّى اللَّهُ عَلَيْهِ وَسَلَّمَ يُنْبَذُ لَهُ الزَّبِيبُ فِي السِّقَاءِ فَيَشْرَبُهُ يَوْمَهُ وَالْغَدَ وَبَعْدَ الْغَدِ فَإِذَا كَانَ مَسَاءُ الثَّالِثَةِ شَرِبَهُ وَسَقَاهُ فَإِنْ فَضَلَ شَيْءٌ أَهَرَاقَهُ

"From Ibn Abbas Radhiyalahu 'anhu, he said,» Rosullullah sallallaahu 'alaihi wa sallam was once made a raisin bath in one vessel, then he drank the marinade that day, also the next day and the next day. On the afternoon of the third day he gave the drink to the others, if there was still something left, then he poured it" [Muslim].

From the hadith above, can be seen that Rosulullah SAW did not drink anything that had been left for more than 3 days. Sinbiotic drinks in this study were fermented for up to 2 days and *Lactobacillus casei* used as a starter in the fermentation process only produced lactic acid as the end result so that the synbiotic drinks were not intoxicating and have a status as halal products.

CHAPTER 5

CLOSING

5.1 Conclusions

Based on the results of research that has been done, it can be concluded that:

1. Fermentation time in the synbiotic drink of mangosteen rind has a significant different effect on pH Level. pH level obtained from the sample ranged at 3.08 to 4.24.
2. Fermentation time in the synbiotic drink of mangosteen rind has significant different effect on antioxidant activity. Synbiotic drink of mangosteen rind have antioxidant activity from weak to strong potential (160.292 – 92.200 ppm).
3. The best sample of synbiotic drink of mangosteen rind based on pH level (4.09) and antioxidant activity (146.409) was the sampel with 24 hours fermentation time.
4. Allah creates various kinds of plants with various benefits in them such as mangosteen. Mangosteen is a source of antioxidants which are compounds that can inhibit the negative effects of free radicals. Allah commands all humans through the Qur'an to always pay attention to food and drinks consumed and provide criteria for foods that are good for human consumption.

5.2 Recommendations

1. Further research needs to identify the type of antioxidants contained in the synbiotic drink of mangosteen rind.
2. Further research is needed to add to the long fermentation time factor to determine the end of limit for the growth of lactic acid bacteria.
3. This study should be used as a reference for further research in developing the function of the mangosteen rind in a variety of food, drink, and pharmaceuticals products.

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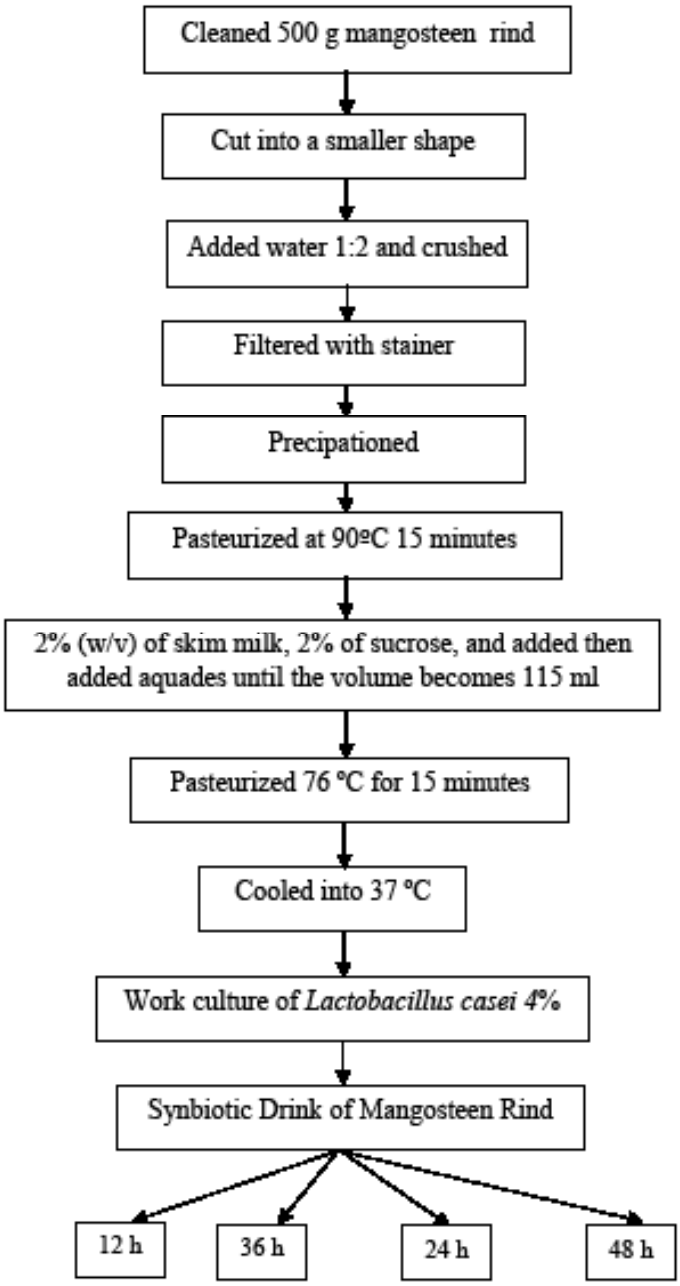
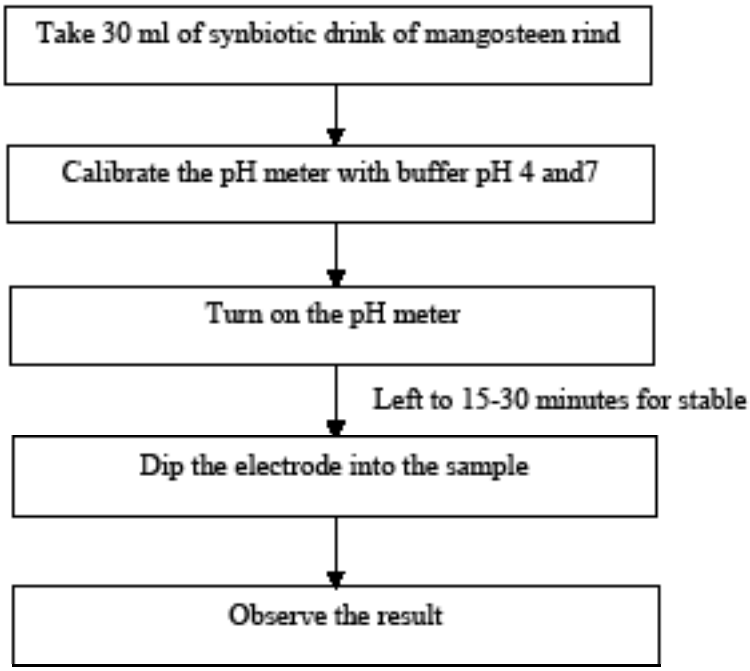
APPENDIX

Appendix 1. Scheme for Making Synbiotic Drink of Mangosteen Rind

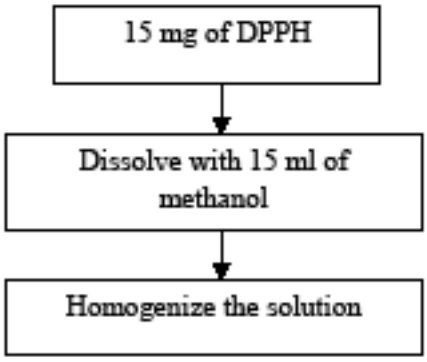


Appendix 2. Scheme for pH Level Analysis



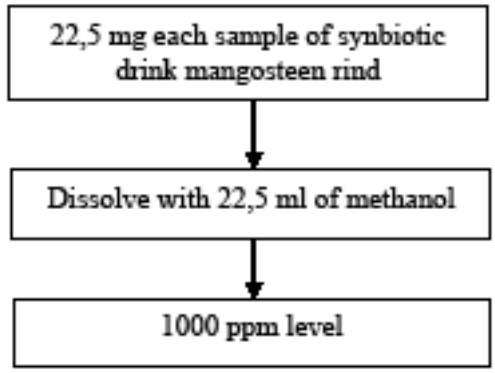
Appendix 3. Shceme of Preparation Solution

A. DPPH Solution

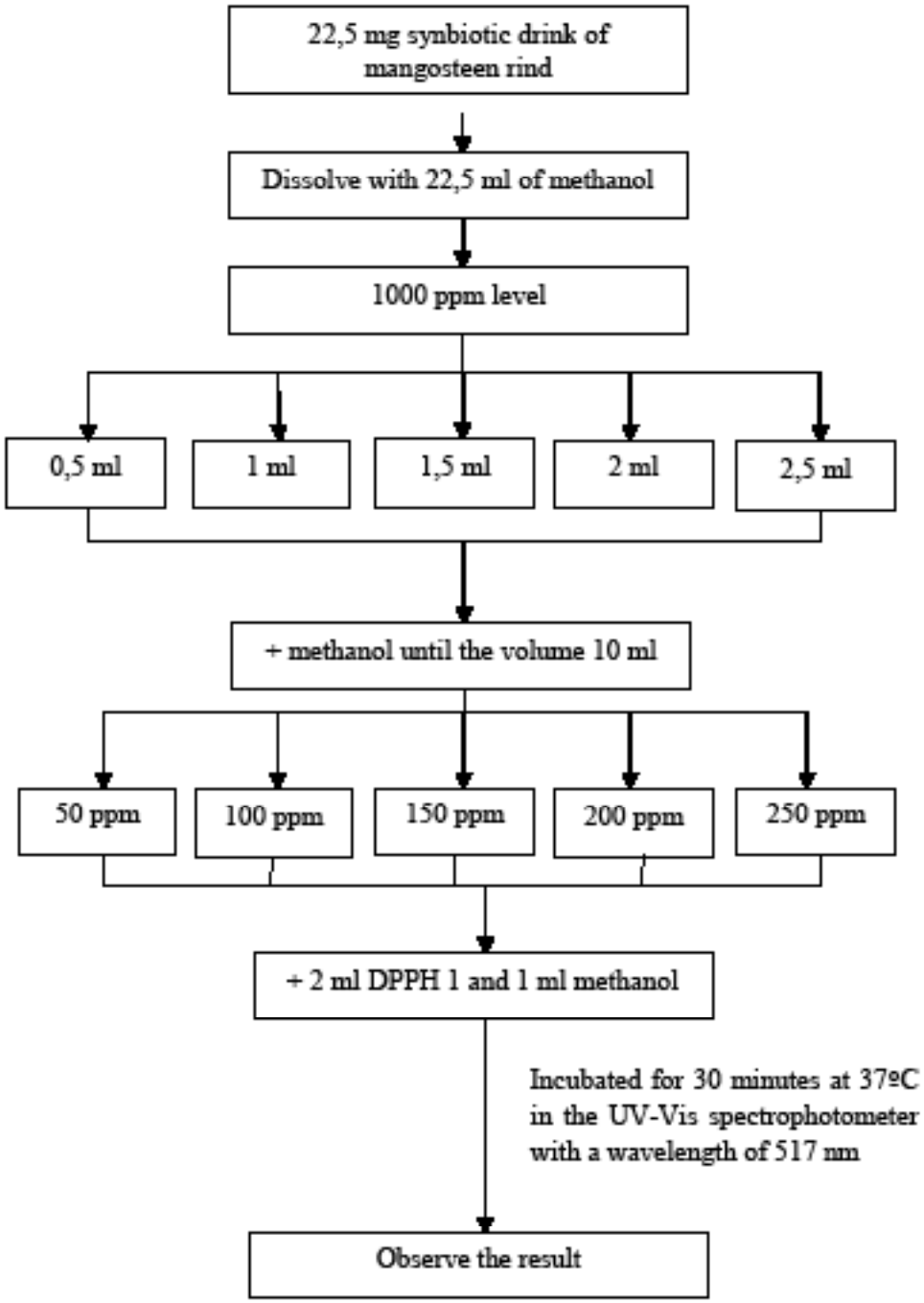


DPPH 1 Solution

B. Test Solution



Appendix 4. Scheme Antioxidant Activity Analysis



Appendix 5. Calculation of Concentration and Sample Dilution

Known : 10 mg sample

10 ml methanol

Asked : ppm ... ?

ppm $= \frac{mg}{L} = \frac{22,5}{0,0225} = 1000$ ppm

50 ppm

V1 x N1 = V2 x N2

V1 x 1000 ppm = 10 ml x 50

V1 $= \frac{500 \text{ ppm.ml}}{1000 \text{ ppm}} = 0,5$ ml

100 ppm

V1 x N1 = V2 x N2

V1 x 1000 ppm = 10 ml x 100

V1 $= \frac{1000 \text{ ppm.ml}}{1000 \text{ ppm}} = 1$ ml

150 ppm

V1 x N1 = V2 x N2

V1 x 1000 ppm = 10 ml x 150

V1 $= \frac{1500 \text{ ppm.ml}}{1000 \text{ ppm}} = 1,5$ ml

200 ppm

V1 x N1 = V2 x N2

V1 x 1000 ppm = 10 ml x 200

V1 $= \frac{2000 \text{ ppm.ml}}{1000 \text{ ppm}} = 2$ ml

250 ppm

V1 x N1 = V2 x N2

V1 x 1000 ppm = 10 ml x 25

V1 $= \frac{250 \text{ ppm.ml}}{1000 \text{ ppm}} = 2,5$ ml

Appendix 6. Data on pH Level of Synbiotic Drinks of Mangosteen Rind

Treatment	Test			Average
	1st Test	2nd Test	3rd Test	
12 hours	4.23	4.25	4.25	4.24
24 hours	4.10	4.08	4.09	4.09
36 hours	3.24	3.20	3.22	3.22
48 hours	3.09	3.06	3.09	3.08

Appendix 7. Data on Antioxidant Activity Value of Synbiotic Drinks of Mangosteen Rind

a.Antioxidant Activity Analysis Data

Fementation Time	Concentration (ppm)	1st Test	2nd Test	3rd Test	Absorbance
12 hours	50	0.645	0.666	0.672	0.825
	100	0.542	0.531	0.538	0.825
	150	0.494	0.473	0.488	0.825
	200	0.268	0.276	0.272	0.825
	250	0.225	0.218	0.231	0.825
24 hours	50	0.637	0.649	0.630	0.825
	100	0.511	0.524	0.519	0.825
	150	0.427	0.448	0.436	0.825
	200	0.244	0.226	0.262	0.825
	250	0.185	0.163	0.197	0.825
36 hours	50	0.572	0.588	0.557	0.825
	100	0.472	0.498	0.446	0.825
	150	0.376	0.401	0.345	0.825
	200	0.193	0.174	0.216	0.825
	250	0.164	0.157	0.166	0.825
48 hours	50	0.524	0.507	0.519	0.825
	100	0.420	0.372	0.412	0.825
	150	0.288	0.271	0.295	0.825
	200	0.096	0.091	0.083	0.825
	250	0.041	0,032	0.049	0.825

b. Antioxidant Activity Analysis 1st test

Fermentation Time	Concentration (ppm)	1st Test	Absorbance	% Inhibition	IC_{50}
12 hours	50	0.645	0.825	21.818	160.055
	100	0.542	0.825	34.303	
	150	0.494	0.825	40.121	
	200	0.268	0.825	67.527	
	250	0.225	0.825	72.691	
24 hours	50	0.637	0.825	22.788	144.993
	100	0.511	0.825	38.061	
	150	0.427	0.825	48.242	
	200	0.244	0.825	70.424	
	250	0.185	0.825	77.576	
36 hours	50	0.572	0.825	30.667	123.906
	100	0.472	0.825	42.788	
	150	0.376	0.825	54.424	
	200	0.193	0.825	76.606	
	250	0.164	0.825	80.121	

48 hours	50	0.524	0.825	36.485	96.248
	100	0.420	0.825	49.091	
	150	0.288	0.825	65.091	
	200	0.096	0.825	88.364	
	250	0.041	0.825	95.006	

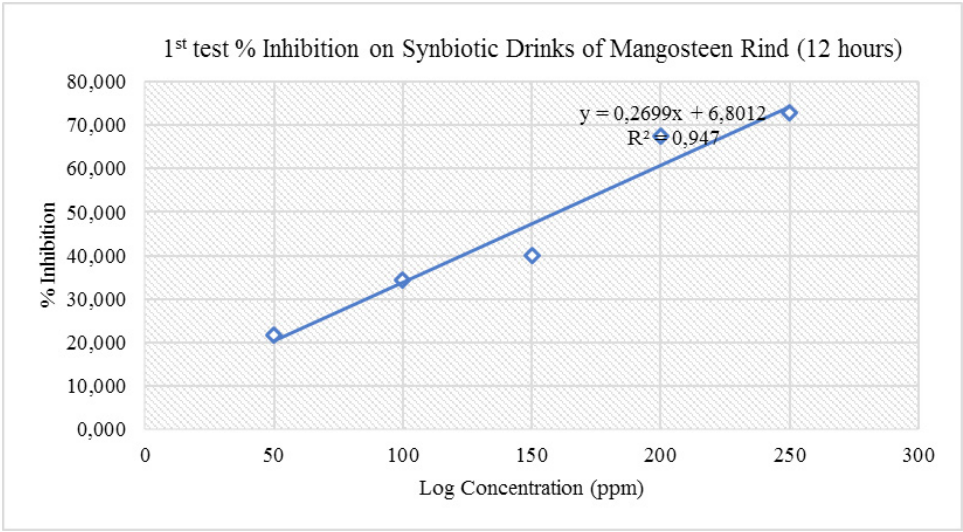
c. Antioxidant Activity Analysis 2nd test

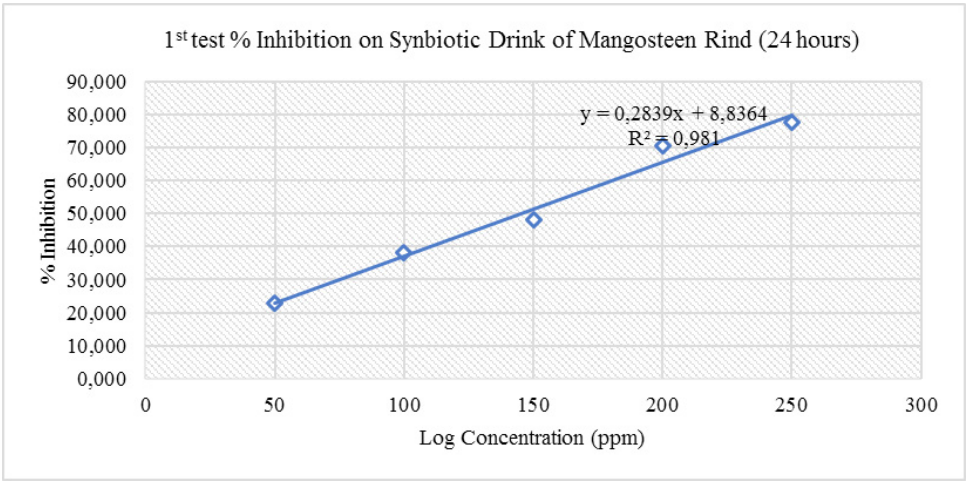
Fermentation Time	Concentration (ppm)	2nd Test	Absorbance	% Inhibition	IC_{50}
12 hours	50	0.666	0.825	19.273	158.796
	100	0.531	0.825	35.636	
	150	0.473	0.825	42.667	
	200	0.276	0.825	66.594	
	250	0.218	0.825	73.576	
24 hours	50	0.649	0.825	21.333	145.859
	100	0.524	0.825	36.485	
	150	0.448	0.825	45.697	
	200	0.226	0.825	72.606	
	250	0.163	0.825	80.242	
36 hours	50	0.588	0.825	28.727	129.391
	100	0.498	0.825	39.636	
	150	0.401	0.825	51.394	
	200	0.174	0.825	78.909	
	250	0.157	0.825	80.970	
48 hours	50	0.507	0.825	38.545	85.856
	100	0.372	0.825	54.909	
	150	0.271	0.825	67.152	
	200	0.091	0.825	89.006	
	250	0.032	0.825	96.121	

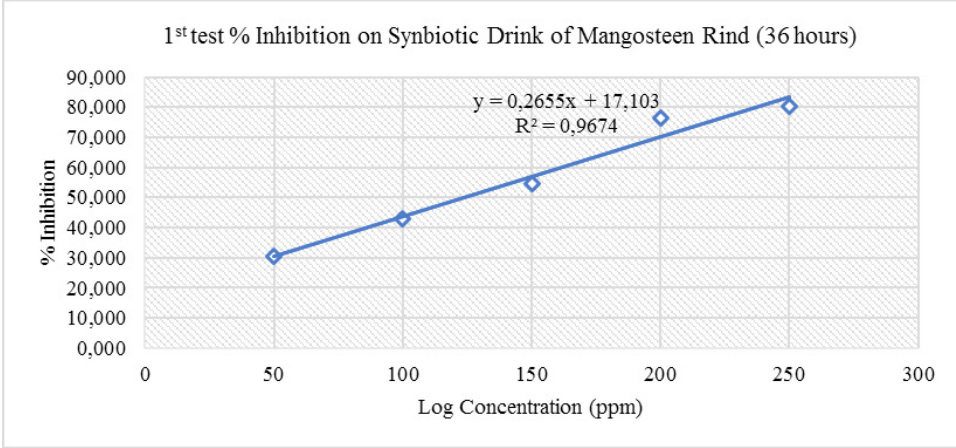
d. Antioxidant Activity Analysis 3rd test

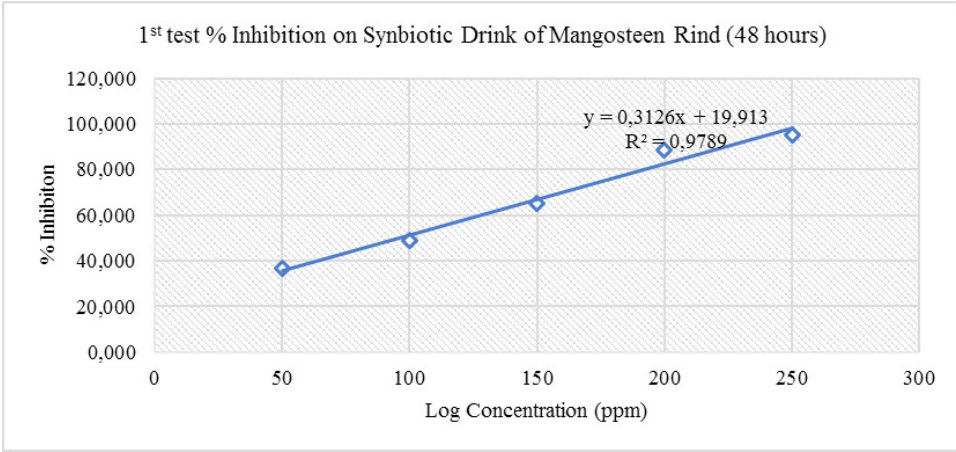
Fermentation Time	Concentration (ppm)	3rd Test	Absorbance	% Inhibition	IC_{50}
12 hours	50	0.672	0.825	18.545	162.025
	100	0.538	0.825	34.788	
	150	0.488	0.825	40.848	
	200	0.272	0.825	67.079	
	250	0.231	0.825	72.000	
24 hours	50	0.630	0.825	23.636	148.375
	100	0.519	0.825	37.091	
	150	0.436	0.825	47.152	
	200	0.262	0.825	68.242	
	250	0.197	0.825	76.121	
36 hours	50	0.557	0.825	32.485	117.158
	100	0.446	0.825	45.939	
	150	0.345	0.825	58.182	
	200	0.216	0.825	73.818	
	250	0.166	0.825	79.879	
48 hours	50	0.519	0.825	37.091	94.496
	100	0.412	0.825	50.061	
	150	0.295	0.825	64.242	
	200	0.083	0.825	89.939	
	250	0.049	0.825	94.061	

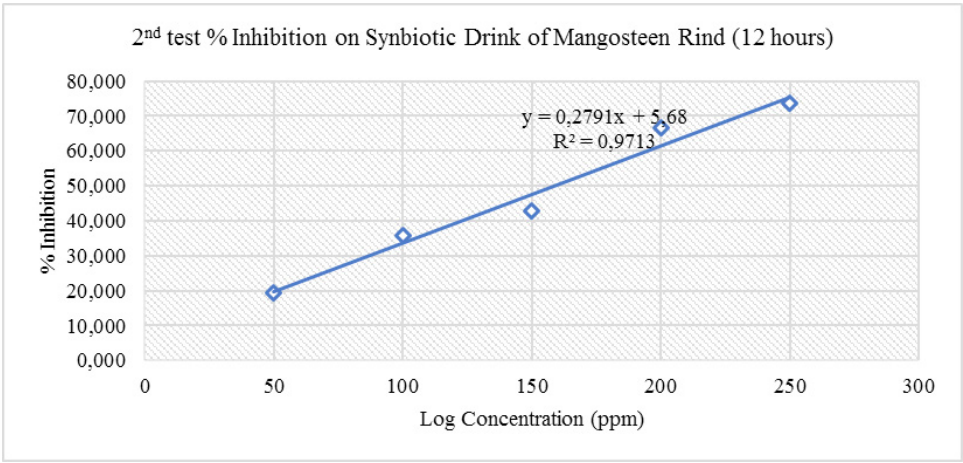
Appendix 8. Graphics

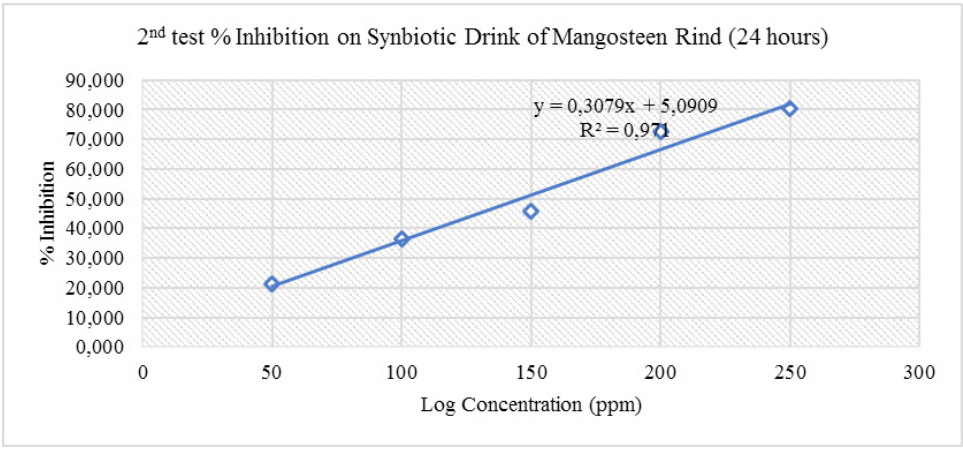


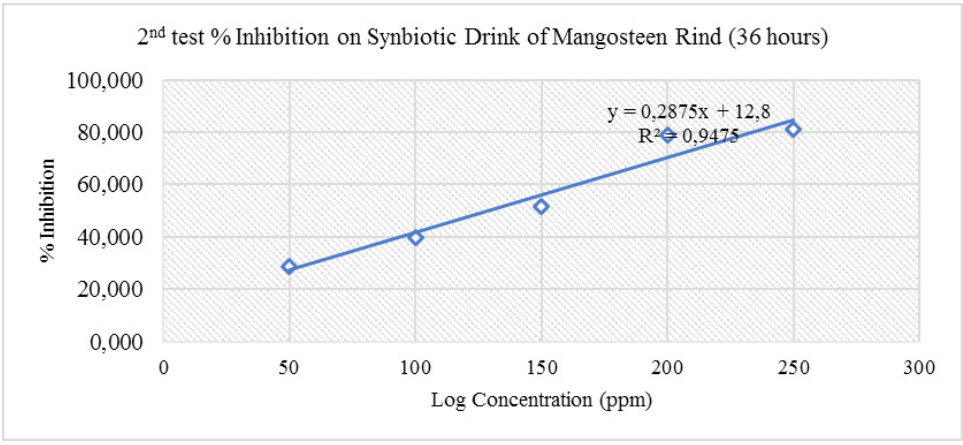


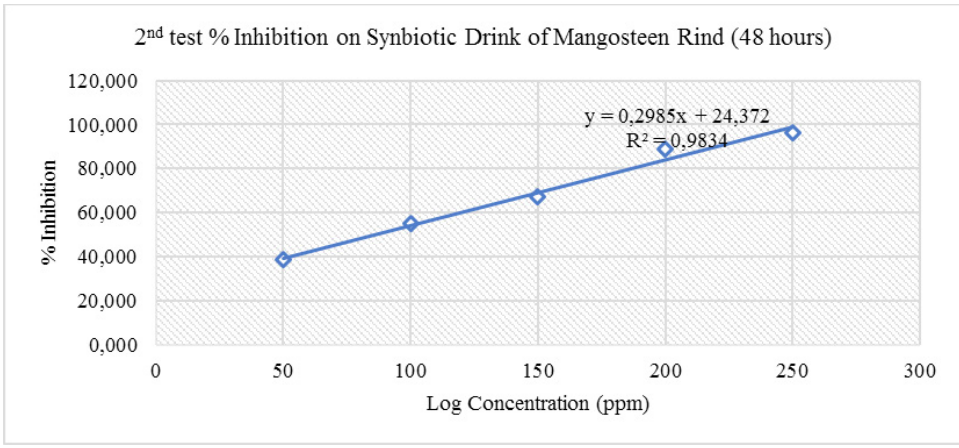

1st test % Inhibition on Synbiotic Drink of Mangosteen Rind (36 hours)
y = 0,2655x + 17,103
R² = 0,9674
% Inhibition
90,000
80,000
70,000
60,000
50,000
40,000
30,000
20,000
10,000
0,000
0
50
100
150
200
250
300
Log Concentration (ppm)

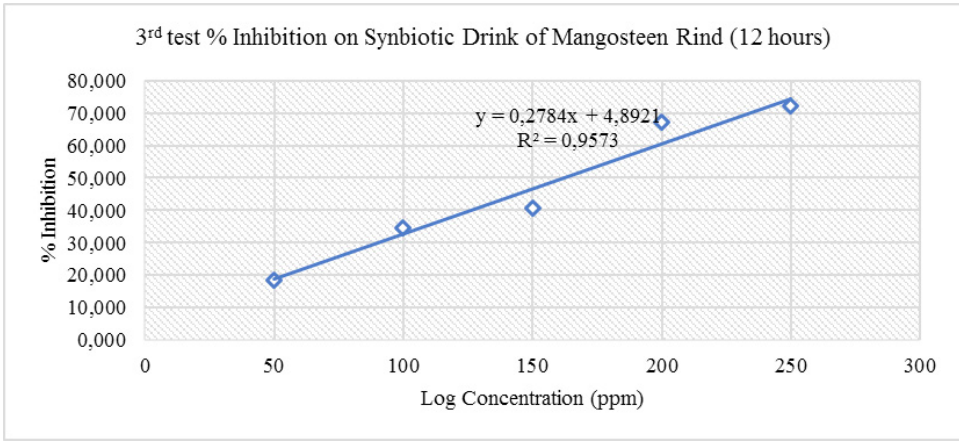

1st test % Inhibition on Synbiotic Drink of Mangosteen Rind (48 hours)
y = 0,3126x + 19,913
R² = 0,9789
% Inhibiton
120,000
100,000
80,000
60,000
40,000
20,000
0,000
0
50
100
150
200
250
300
Log Concentration (ppm)

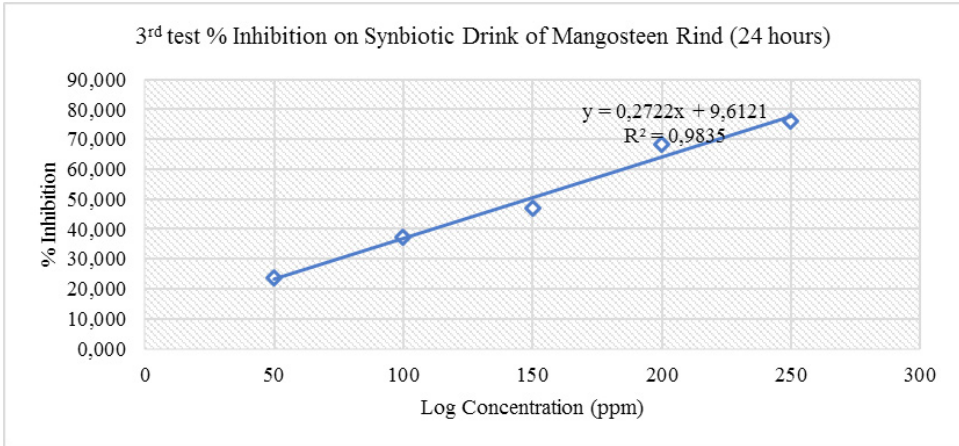

2nd test % Inhibition on Synbiotic Drink of Mangosteen Rind (12 hours)
y = 0,2791x + 5,68
R² = 0,9713
% Inhibition
Log Concentration (ppm)
80,000
70,000
60,000
50,000
40,000
30,000
20,000
10,000
0,000
0
50
100
150
200
250
300

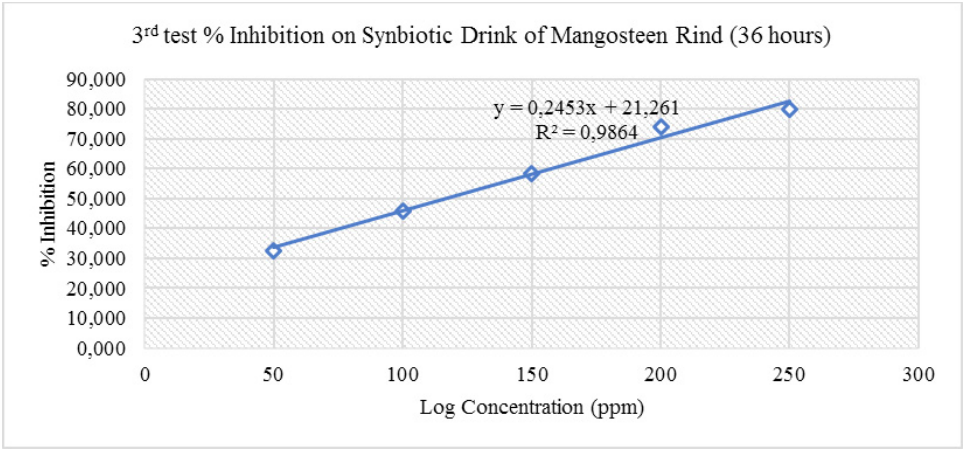

2nd test % Inhibition on Synbiotic Drink of Mangosteen Rind (24 hours)
y = 0,3079x + 5,0909
R² = 0,971
% Inhibition
Log Concentration (ppm)
90,000
80,000
70,000
60,000
50,000
40,000
30,000
20,000
10,000
0,000
0
50
100
150
200
250
300

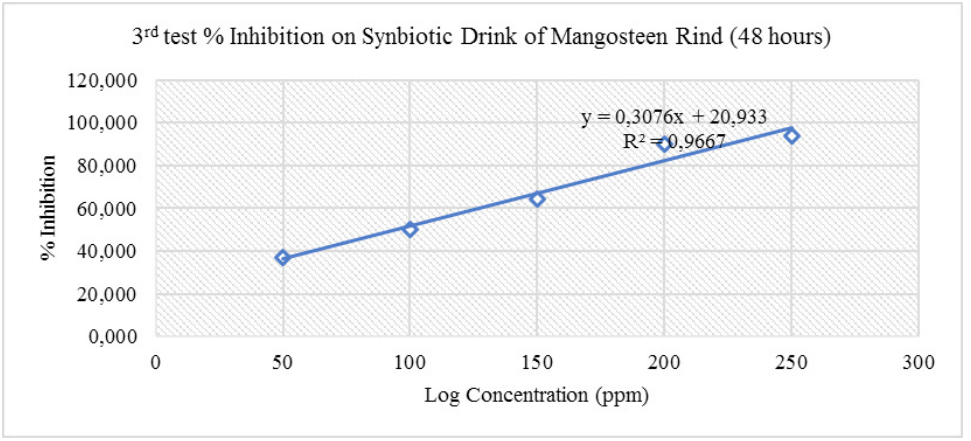

2nd test % Inhibition on Synbiotic Drink of Mangosteen Rind (36 hours)
y = 0,2875x + 12,8
R² = 0,9475
% Inhibition
Log Concentration (ppm)
100,000
80,000
60,000
40,000
20,000
0,000
0
50
100
150
200
250
300


2nd test % Inhibition on Synbiotic Drink of Mangosteen Rind (48 hours)
y = 0,2985x + 24,372
R² = 0,9834
% Inhibition
Log Concentration (ppm)
120,000
100,000
80,000
60,000
40,000
20,000
0,000
0
50
100
150
200
250
300


3rd test % Inhibition on Synbiotic Drink of Mangosteen Rind (12 hours)
y = 0,2784x + 4,8921
R² = 0,9573
% Inhibition
Log Concentration (ppm)
80,000
70,000
60,000
50,000
40,000
30,000
20,000
10,000
0,000
0
50
100
150
200
250
300


3rd test % Inhibition on Synbiotic Drink of Mangosteen Rind (24 hours)
y = 0,2722x + 9,6121
R² = 0,9835
% Inhibition
Log Concentration (ppm)
90,000
80,000
70,000
60,000
50,000
40,000
30,000
20,000
10,000
0,000
0
50
100
150
200
250
300





Appendix 9. IC_{50} Calculation

1. 12 hours Fermentation Time
   a. 1st test

      y = 0,2699x + 6,812

      50 – 6,812= 0,2699

      43,188 = 0,2699

      x $= \frac{43,188}{0,2699}$ =160,014

   b. 2nd test

      y = 0,2791x + 5,68

      50 – 5,68 = 0,2791

      44,32 = 0,2791

$$x = \frac{44,32}{0,2791} = 158,796$$

c. 3rd test

y = 0,2784x + 4,892

50 – 4,892 = 0,2784

45,108 = 0,2784

$$x = \frac{45,108}{0,2784} = 162,025$$

2. 24 hours Fermentation Time

a. 1st test

y = 0,2839x + 8,8364

50 – 8,8364= 0,2839

41, 1636 = 0,2839

$$x = \frac{41,1636}{0,2839} = 144,993$$

b. 2nd test

y = 0,3079x + 5,0909

50 – 5,0909= 0,3079

44,9091 = 0,3079

$$x = \frac{44,9091}{0,3079} = 145,856$$

c. 3rd test

y = 0,2722x + 9,6121

50 – 9,6121= 0,2722

40,3879 = 0,2722

$$x = \frac{40,3879}{0,2722} = 148,375$$

3. 36 hours Fermentation Time

a. 1st test

y = 0,2655x + 17,103

50 – 17,103 = 0,2655

32,897 = 0,2655

$$x = \frac{32,897}{0,2655} = 123,905$$

b. 2nd test

$y = 0,2875x + 12,8$

$50 - 12,8 = 0,2875$

$37,2 = 0,2875$

$$x = \frac{37,2}{0,287} = 129,616$$

c. 3rd test

$y = 0,2453x + 21,261$

$50 - 21,261 = 0,2453$

$28,739 = 0,2453$

$$x = \frac{28,739}{0,2453} = 117,158$$

4. 48 hours Fermentation Time

a. 1st test

$y = 0,3126x + 19,913$

$50 - 19,913 = 0,3126$

$30,087 = 0,3126$

$$x = \frac{30,087}{0,3126} = 96,247$$

b. 2nd test

$y = 0,2985x + 24,372$

$50 - 24,372 = 0,2985$

$25,628 = 0,2985$

$$x = \frac{25,628}{0,2985} = 85,855$$

c. 3rd test

$y = 0,3076x + 20,933$

$50 - 20,933 = 0,3076$

$29,067 = 0,3076$

$$x \qquad \frac{29,067}{0,3076} = 94,496$$

Appendix 10. SPSS Statistical Analysis on The Effect of Fermentation Time on pH Value and Antioxidant Activity Synbiotic Drink of Mangosteen Rind

a. Analysis of The pH Value of Synbiotic Drink of Mangosteen Rind

NPar Tests

Descriptives

result

	N	Mean	Std. Deviation	Minimum	Maximum
12 hours	3	4.2433	.01155	4.23	4.25
24 hours	3	4.0900	.01000	4.08	4.10
36 hours	3	3.2200	.02000	3.20	3.24
48 hours	3	3.0800	.01732	3.06	3.09
Total	12	3.6583	.53660	3.06	4.25

One-Sample Kolmogorov-Smirnov Test

		Unstandardized Residual
N		12
Normal Parameters[a]	Mean	.0000000
	Std. Deviation	.16947155
Most Extreme Differences	Absolute	.143
	Positive	.143
	Negative	-.135
Kolmogorov-Smirnov Z		.497
Asymp. Sig. (2-tailed)		.966

a. Test distribution is Normal.

x

Oneway

Descriptives

result

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
12 hours	3	4.2433	.01155	.00667	4.2146	4.2720	4.23	4.25
24 hours	3	4.0900	.01000	.00577	4.0652	4.1148	4.08	4.10
36 hours	3	3.2200	.02000	.01155	3.1703	3.2697	3.20	3.24
48 hours	3	3.0800	.01732	.01000	3.0370	3.1230	3.06	3.09
Total	12	3.6583	.53660	.15490	3.3174	3.9993	3.06	4.25

Test of Homogeneity of Variances

result

Levene Statistic	df1	df2	Sig.
.621	3	8	.621

ANOVA

result

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.165	3	1.055	4.522E3	.000
Within Groups	.002	8	.000		
Total	3.167	11			

Post Hoc Tests

Multiple Comparisons

result

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
12 hours	24 hours	.15333*	.01247	.000	.1134	.1933
	36 hours	1.02333*	.01247	.000	.9834	1.0633
	48 hours	1.16333*	.01247	.000	1.1234	1.2033
24 hours	12 hours	-.15333*	.01247	.000	-.1933	-.1134
	36 hours	.87000*	.01247	.000	.8301	.9099
	48 hours	1.01000*	.01247	.000	.9701	1.0499
36 hours	12 hours	-1.02333*	.01247	.000	-1.0633	-.9834
	24 hours	-.87000*	.01247	.000	-.9099	-.8301
	48 hours	.14000*	.01247	.000	.1001	.1799
48 hours	12 hours	-1.16333*	.01247	.000	-1.2033	-1.1234
	24 hours	-1.01000*	.01247	.000	-1.0499	-.9701
	36 hours	-.14000*	.01247	.000	-.1799	-.1001

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

Homogeneous Subsets

result

Tukey HSD

treatment	N	Subset for alpha = 0.05			
		1	2	3	4
48 hours	3	3.0800			
36 hours	3		3.2200		
24 hours	3			4.0900	
12 hours	3				4.2433
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

b. Analysis of Antioxidant Activity of Synbiotic Drink of Mangosteen Rind

Tests of Normality

	treatment	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
aa	12hours	.224	3	.	.984	3	.759
	24hours	.290	3	.	.926	3	.472
	36hours	.194	3	.	.997	3	.887
	48hours	.327	3	.	.872	3	.302

a. Lilliefors Significance Correction

Oneway

Test of Homogeneity of Variances

aa

Levene Statistic	df1	df2	Sig.
2.217	3	8	.164

Descriptives

aa

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
12hours	3	1.6029E2	1.62795	.93990	156.2426	164.3307	158.79	162.02
24hours	3	1.4640E2	1.75663	1.01419	142.0396	150.7670	144.99	148.37
36hours	3	1.2348E2	6.12615	3.53694	108.2648	138.7012	117.16	129.39
48hours	3	92.2000	5.56346	3.21207	78.3796	106.0204	85.86	96.25
Total	12	1.3059E2	27.16482	7.84181	113.3335	147.8530	85.86	162.02

ANOVA

aa

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7968.765	3	2656.255	143.160	.000
Within Groups	148.436	8	18.554		
Total	8117.201	11			

Multiple Comparisons

aa

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
12hours	24hours	13.88333*	3.51705	.018	2.6205	25.1462
	36hours	36.80367*	3.51705	.000	25.5408	48.0665
	48hours	68.08667*	3.51705	.000	56.8238	79.3495
24hours	12hours	-13.88333*	3.51705	.018	-25.1462	-2.6205
	36hours	22.92033*	3.51705	.001	11.6575	34.1832
	48hours	54.20333*	3.51705	.000	42.9405	65.4662
36hours	12hours	-36.80367*	3.51705	.000	-48.0665	-25.5408
	24hours	-22.92033*	3.51705	.001	-34.1832	-11.6575
	48hours	31.28300*	3.51705	.000	20.0202	42.5458
48hours	12hours	-68.08667*	3.51705	.000	-79.3495	-56.8238
	24hours	-54.20333*	3.51705	.000	-65.4662	-42.9405
	36hours	-31.28300*	3.51705	.000	-42.5458	-20.0202

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

aa

Tukey HSD

treatment	N	Subset for alpha = 0.05			
		1	2	3	4
48hours	3	92.2000			
36hours	3		1.2348E2		
24hours	3			1.4640E2	
12hours	3				1.6029E2
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.