THESIS

THE IMPLICATIONS OF SCOBY (SYMBIOTIC CULTURE OF BACTERIA AND YEAST) VARIATION ON THE QUALITIES OF ARABICA AND ROBUSTA CASCARA KOMBUCHA

Meysin Anjliany
05031181722046

AGRICULTURAL PRODUCT TECHNOLOGY STUDY PROGRAM
DEPARTMENT OF AGRICULTURAL CULTIVATION
FACULTY OF AGRICULTURE
SRIWIJAYA UNIVERSITY
2021

THESIS

THE IMPLICATIONS OF SCOBY (SYMBIOTIC CULTURE OF BACTERIA AND YEAST) VARIATION ON THE QUALITIES OF ARABICA AND ROBUSTA CASCARA KOMBUCHA

This thesis was written to fulfill one of the requirements to accomplish a Bachelor's Degree in Agriculture Product Technology Study Program at the Faculty of Agriculture, Sriwijaya University

Meysin Anjliany
05031181722046

AGRICULTURAL PRODUCT TECHNOLOGY STUDY PROGRAM
DEPARTMENT OF AGRICULTURAL CULTIVATION
FACULTY OF AGRICULTURE
SRIWIJAYA UNIVERSITY
2021

APPROVAL SHEET

THE IMPLICATIONS OF SCOBY (SYMBIOTIC CULTURE OF BACTERIA AND YEAST) VARIATION ON THE QUALITIES OF ARABICA AND ROBUSTA CASCARA KOMBUCHA

THESIS

This thesis was written to fulfill one of the requirements to accomplish a Bachelor's Degree in Agriculture Product Technology Study Program at the Faculty of Agriculture, Sriwijaya University

By : Meysin

Anjliany
05031181722046

Indralaya, September 2021
Advisor

Dr. Merynda Indriyani Syafutri, S.TP., M.Si
NIP. 198203012003122002

Certified by,
Dean of the Faculty of Agriculture

Dr. Ir. A. Muslim, M. Agr
NIP. 196412291990011001

SUMMARY

MEYSIN ANJLIANY. The Implications of SCOBY (Symbiotic Culture of Bacteria And Yeast) Variation on The Qualities of Arabica And Robusta Cascara Kombucha (Supervised by **MERYNDA INDRIYANI SYAFUTRI**).

This research aimed to determine the effect of coffee peel type and variation of SCOBY concentration on total phenol, total titrated acid, total dissolved solid, pH and total plate count of cascara kombucha. This research was conducted at Agricultural Product Processing Laboratory, Agricultural Product Chemical Laboratory and Agricultural Product Microbiology Laboratory, Agricultural Technology Department, Faculty of Agriculture, Sriwijaya University, Indralaya, South Sumatera.This research used Completely Randomized Factorial Design with two treatment factors and repeated three times. The first factor was coffee peel type (arabica and robusta) and the second factor was SCOBY concentration variation (5%, 10% and 15%). Parameters observed were total phenol, total titrated acid, total dissolved solid, pH and total plate count.The results showed that coffee peel type, SCOBY concentration variation, and interaction of coffee peel type and SCOBY concentration variation had significant effects on total titrated acid and total dissolved solid cascara kombucha. The best treatment was selected based on the closest value to quality standard corresponded to Kombucha Brewers International (2021). Cascara kombucha with robusta coffee peels and SCOBY concentration 10% was chosen as the best treatment with total phenol 93.18 GAE/mL, total titrated acid 0.82%, total dissolved solid 3.80°Brix, pH 4.21, and TPC 5.04 x 10^7 CFU/mL.

INTEGRITY STATEMENT

The undersigned below:

Name	: Meysin Anjliany
Student Number	: 05031181722046
Title	: The Implications of Scoby (Symbiotic Culture of Bacteria and Yeast) Variation on The Qualities of Arabica and Robusta Cascara Kombucha

Declare that all data and information contained in this thesis are the result of my own research activities under the supervision of my advisors, unless the sources are clearly mentioned. If in the future found any element of plagiarism in this thesis, then I am willing to accept academic sanctions from Sriwijaya University.

Thus, I make this statement consciously and without coercion from any party.

Indralaya, November 2021

(Meysin Anjliany)

BIOGRAPHY

Meysin Anjliany—the writer's name, is the first child of Mr. Rizalasih and Mrs. Andrita who was born on May 4, 2000 in Prabumulih. When writing this curriculum vitae, the writer had completed elementary school education at SD Negeri 14 Kota Prabumulih in 2011, junior high school at SMP Negeri 1 Prabumulih in 2014 and high school at SMA Negeri 1 Prabumulih in 2017. The writer succeeded in holding active student status. Agricultural Products Technology Study Program, Faculty of Agriculture, Sriwijaya University as of August 2017 since it was declared to have passed the National Selection to Enter State Universities.

While studying at Sriwijaya University, the writer underwent an AIMS (Asean International Mobility Student) overseas student exchange program for one semester from August to December 2019 at Kasetsart University, Bangkok, Thailand. Additionally, the writer also carried out Real Work Lectures (KKN) in Tapus Village, Lembak District, Muara Enim Regency, South Sumatra from December 2020 to January 2021 and Field Practice at Robbani Snack, Pringsewu, Lampung from August to September 2021.

The writer plays an active role in organizational activities on and off campus, including the Agricultural Technology Student Association (HIMATETA) Sriwijaya University since 2017 and the Indonesian Food Care Student Association (HMPPI) since 2018. Since 2018, the writer has been registered as a practicum assistant at the General Chemistry Laboratory, Laboratory Joint Foundation, Sriwijaya University and Analytical Chemistry practicum assistant in 2019, Hygiene and Sanitation practicum in 2020, General Microbiology practicum in 2020 and Food Processing Microbiology practicum in 2021.

ACKNOWLEDGEMENT

Bismillah. All praise to Allâh, the Lord of the Worlds. All praise and thanks are due to Allah Subhanahu wa ta'ala, Lord of the worlds, because of His mercy, blessings and grace, the writer was able to complete the process of writing this thesis as well as possible. Shalawat and greetings are sent to the great prophet Muhammad sallallaahu 'alaihi wa sallam and the people who are in His path. During carrying out the research until the completion of this thesis, the writer got help, guidance, support from various parties. So on this occasion, the writer would like to thank:

1. T h e Dean of the Faculty of Agriculture, Sriwijaya University.
2. The Chair and Secretary of the Department of Agriculture Technology, Faculty of Agriculture, Sriwijaya University.
3. The Coordinator of Agricultural Product Technology Study Program, Agricultural Technology Department, Faculty of Agriculture.
4. **Mrs. Dr. Ir. Hj. Tri Wardani Widowati, M.P** as the Academic Supervisor and Field Practice Supervisor who has taken the time, provides direction, advice, suggestions, solutions, motivation, guidance, enthusiasm and prayer to the writer.
5. **Mrs. Dr. Merynda Indriyani Syafutri, S.TP., M.Si** as the thesis supervisor who has taken the time, provides direction, advice, suggestions, solutions, motivation, guidance and enthusiasm to the writer.
6. **Mr. Dr. Rer. Nat. Ir. Agus Wijaya, M.Sc** as the Discussion of the Thesis Papers and Examiners who have provided input, direction, prayer and guidance to the writer.
7. **Mrs. Dr. Ir. Parwiyanti, M.P** who has given a lot of input during the thesis process.
8. The Lecturers of Agricultural Technology Department who have educated, share knowledge and motivation.
9. Academic Administration Staff Department of Agricultural Technology (**Kak Jhon** and **Mbak Desi**) and laboratory staff majoring in Agricultural

Technology (**Mbak Hafsah, Mbak Elsa, Mbak Lisma** and **Mbak Tika**) for all the assistance and convenience provided.

10. Both of my parents **Rizalasih** and **Yulis,** who have given prayers, beliefs, advice, motivation and enthusiasm. Never pressure to graduate as soon as possible and never set the value to be achieved. Thank you for the full trust that has been given.
11. Aunties, **Ibung Leni Marlen** and **Ibung Siti Aslah** who always provide support both material and moral support.
12. Uncle, **Wak Efran Murod**, who has provided a lot of assistance in the form of moral and material support.
13. A large family that cannot be mentioned one by one, thank you for the advice, enthusiasm and prayer that is always accompanying.
14. **Kosan Squad: Desmianti, Lailatul Nazmi, Byita Puspaningrum, Mbak Devi Mawarni,** and **Deiska Centrilisyana,** my best friends, who always accompany in difficult times, share stories and laughter. Thank you for your lecture life feels exciting.
15. **Ferliansyah, Tania Fazalia** and **Sayidil Tohari,** my friends and Abangku who are always in every trace of lectures. Thank you for listening to all complaints. Thank you for all the stories, questions and simple stories that help mentally stay healthy.
16. **Ismi Trifiamola, Tri Adelti,** and **Untanti Rasuanti, friends** since new students. Thank you for giving up more than four years of lecture.
17. **Dewi Ananda Apriani,** thank you always divide the judicium preparation file and become a partner to prepare starting from the semhas.
18. **Kak Alrevansyah,** my brother, who has introduced to international competition, always supports and provides direction to develop himself better.
19. Kiyowo Team: **Ramadhannie Fitra** and **Ilham Akbar,** my younger siblings. People call it 'busy' but we call it 'spending time in a great way, while recording great memory, to achieve great results'. So thanks to have you guys in this journey.

20. My Family of Agricultural Product Technology 2017 Indralaya that cannot be mentioned one by one.

21. Myself, who had made it through all the falling points, the crisis of self - confidence, and all adversity so that it could reach this point, so far, with all the achievements. Thank you for struggling happily and enjoying every process.

Hopefuly, this thesis can give useful contribution to all of us in the development of science.

CONTENTS

Page

OUTLINE x

LIST OF TABLES xii

LIST OF FIGURES xiii

CHAPTER 1. INTRODUCTION 1

1.1. Background 1

1.2. Objective 3

1.3. Hipothesis 3

CHAPTER 2. LITERATURE REVIEW 4

2.1. Coffee 4

2.1.1. Characteristics and Content of Arabica 4

2.1.2. Characteristics and Content of Robusta 5

2.2. Cascara 6

2.3. Kombucha 8

2.3.1. Kombucha Chemical Ingredients 9

2.4. SCOBY 9

CHAPTER 3. RESEARCH METHODOLOGY 11

3.1. Place and Time 11

3.2. Tools and Materials 11

3.3. Research Method 11

3.4. Data Analysis 12

3.5. Statistical Data Analysis 12

3.6. Procedures 14

3.7. Parameters 15

3.7.1. Total Polyphenol Test 15

3.7.2. Total Titratable Acidity (TTA) Test 15

3.7.3. Total Dissolved Solids 17

3.7.4. pH Analysis 17

3.7.5. Total Plate Number Test 17

CHAPTER 4. RESULTS AND DISCUSSION 18
4.1. Total Phenol 18
4.2. Total Acid Titrated 20
4.3. Total Dissolved Solids 24
4.4. pH Analysis 27
4.5. Total Plate Number 29
CHAPTER 5. CONCLUSIONS AND SUGGESTIONS 32
5.1. Conclusions 32
5.2. Suggestions 32
REFERENCES 33

LIST OF TABLES

Page

Table 2.1. The antioxidant content of cascara 7

Table 2.2. Chemical content in 120 mL of kombucha 9

Table 3.1. List of Factorial Completely Randomized Design Variance Analysis (FCRD) 13

Table 4.1. The result of further test HSD 5% the effect of coffee husk type on the total phenol of kombucha cascara 19

Table 4.2. Results of further test HSD 5% the effect of different types of coffee treatment factors on total acid titrated kombucha cascara 21

Table 4.3. Results of further test HSD 5% the effect of treatment factors increasing the concentration of SCOBY on the total titrated acid of kombucha cascara 22

Table 4.4. Results of further test HSD 5% the effect of the interaction of the two treatment factors on the total titrated acid of kombucha cascara 23

Table 4.5. Results of further test of 5% HSD the effect of different types of coffee treatment factors on the total dissolved solids of kombucha cascara 25

Table 4.6. Results of further test HSD 5% the effect of treatment factors increasing the concentration of SCOBY on the total dissolved solids of kombucha cascara 26

Table 4.7. The result of further test HSD 5% interaction effect of coffee skin type and variation of SCOBY concentration on the total dissolved solids of kombucha cascara 26

Table 4.8. The results of the HSD further test 5% the effect of the coffee skin type treatment factor on the pH value of kombucha cascara 29

LIST OF FIGURES

Page

Figure 2.1. Coffee Structure 4
Figure 2.2. Arabica 4
Figure 2.3. Robusta 5
Figure 2.4. Cascara 6
Figure 2.5. Kombucha 8
Figure 2.6. SCOBY 9
Figure 4.1. The average total phenol value (µg/mL) of kombucha cascara 18
Figure 4.2. The average total titrated acid value (%) kombucha cascara 21
Figure 4.3. Average value of total dissolved solids of kombucha cascara …… 24
Figure 4.4. Average pH value of kombucha cascara 28
Figure 4.5. The average total plate number (CFU/mL) of kombucha cascara 30

CHAPTER 1
INTRODUCTION

1.1. Background

Coffee is the sub-sector of the highest natural resource base as well as a national strategic plantation commodity. In 2018, Indonesia became the fourth largest coffee exporter in the global market after Brazil, Vietnam and Colombia by controlling 6.06% of total world coffee exports (International Coffee Organization, 2019). Arabica (*Coffea arabica*) and robusta (*Coffea robusta*) are the two most widely cultivated coffee species in Indonesia (Sulistyaningtyas, 2017; Villanueva et al., 2011). Arabica is the best coffee in taste and quality. It has a strong and slightly sour taste with a caffeine content of 1-1.3%. However, Arabica is very susceptible to *Hemelia vasstatrik*, a leaf rust disease (Panggabean, 2011). Among all types of coffee, Robusta has the best resistance to leaf rust disease. It has a taste similar to chocolate, bitter than arabica, slightly sour and has a characteristic sweet smell (Prawosto et al., 2010).

The area of Indonesian coffee plantations reaches 1,227,728 hectares with coffee production of 637,539 tons per year (Muzaifa *et al.*, 2019). According to the Director General of Plantations (2017), the region with the highest coffee production in Indonesia is South Sumatra, which has an area of 250,171 hectares with 11,048 tons of robusta plantation. Meanwhile, the largest arabica producing area is Aceh with Arabica production of 47,378 tons per year and an area of 121,060 hectares.

The trend of coffee-based drinks tends to be stable and increasing. The increase in the popularity of coffee-based drinks has an impact on increasing the productivity of the national coffee commodity. The mushrooming of coffee processing also has an impact on the mountains of coffee waste. Muzaifa et al. (2019) stated that coffee waste was the non-bean part obtained from the results of coffee processing. Aini *et al*. (2019 added that the coffee waste consists of fruit rind (45%), mucilage (10%) and seed coat (5%). Generally, coffee rind is used in the manufacture of fertilizer. In addition, coffee rind is also used as animal feed material after going through quality improvement with *Phanerochaete chrysosporium* fermentation method.

Coffee husk waste is a potential material that can be optimized as a producer of caffeine, polyphenols, bioethanol (Bonilla-Hermosa et al., 2014), antioxidants and antimicrobials (Jimenez-Zamora et al., 2015). Coffee husk has many benefits including being able to ward off free radicals, protect the stomach and is good for giving a firming effect to the skin. The ability to ward off free radicals from coffee skin is suitable for preventing the growth of cancer cells and can improve the immune system. The active compounds contained in coffee husk include phenols, tannins (1.8-8.56%), pectin (6.5%), caffeine (1.3%), chlorogenic acid (2.6%), caffeic acid. (1.6%), and total anthocyanins (cyanidine, delpinidin, cyanidin 3-glycoside, delpinidine 3-glycoside, and pelargonidin 3-glycoside) 43% (Sumihati and Widiyanto, 2011).

The high intensity of coffee husk waste generated from coffee processing creates the potential for the development of the derivative products of coffee husk, including cascara. Cascara is the dried skin of coffee and is usually processed into refreshing drinks such as tea (Pabari, 2014). Cascara steeping has a blend of fruity flavors and blends of strawberry, raisin (Sawab et al., 2017) rose, cherry, mango and tobacco aromas (Muzaifa et al., 2019). The caffeine content of cascara is 226 mg caffeine/L, while the dominant phenolic compounds are protocatechuic and chlorogenic acid which are 85.0 and 69.6 mg/L (Heeger et al., 2017).

One of the functional drinks that are currently favored by the public, especially healthy lifestyle activists, is kombucha. Kombucha is a beverage fermented from tea liquid containing sugar by a consortium of microorganisms from the acetic acid bacteria group, namely *Acetobacter xylinum* and the yeast *Saccharomyces sp.* (Yanti *et al*., 2020) with a fermentation process carried out for 1-2 weeks (Sun *et al*., 2015). This symbiotic culture between bacteria and yeast is known as SCOBY or symbiotic culture of bacteria and yeast. The amount of addition of SCOBY will affect the production of organic acids in kombucha. Based on Urbahillah (2018), 10% (w/v) SCOBY and 10% (w/v) added sugar are the optimum amounts for making kombucha. This treatment will produce the highest acetic acid, which is about 0.78%. Optimum results are obtained because the availability of nutrients is proportional to the number of microorganisms.

According to Viviandri *et al.* (2015), the bacteria involved in the kombucha fermentation process are acetic acid bacteria and lactic acid bacteria. This shows that kombucha has a potential as a probiotic drink. The fermentation process will cause changes in physical and chemical properties which include sugar, alcohol content, pH and antioxidant levels (Nguyen et al., 2015). This change was caused by Saccharomyces sp. which breaks down glucose into ethanol while Acetobacter xylinum will oxidize ethanol to acetic acid (Ayuratri and Kusnadi, 2017). The fermentation process will be able to increase the total polyphenols due to microbial enzymatic activity which can free bound polyphenolic compounds so that more will be detected (Zubaidah *et al*., 2012). Fermentation will also increase the total titrated acid caused by the breakdown of sugar compounds into organic acids. An increase in the total titrated acid will have an effect on a decrease in pH.

Research on kombucha with tea-based ingredients has been widely carried out, even now the manufacture of kombucha with raw materials other than tea such as coffee beans (Rahayu, 2006), rosella flowers (Nainggolan, 2009) and soursop leaves (Falahuddin, 2017). However, the manufacture of kombucha with cascara as the raw material has not been carried out even though cascara is known to be rich in benefits. The manufacture of kombucha cascara is expected to be able to produce a diversified product of processed coffee skin that has functional properties. The use of different types of coffee and various concentrations of SCOBY (symbiotic culture of bacteria and yeast) is thought to affect the chemical and microbiological characteristics of kombucha cascara. A total lactic acid bacteria test is also required to claim kombucha as a probiotic functional drink.

1.2. Objective

The objective of this study was to determine the effect of the type of coffee (Coffea arabica and Coffea robusta) and the concentration of SCOBY (symbiotic culture of bacteria and yeast) on the chemical and microbiological characteristics of kombucha cascara.

1.3. Hypothesis

The use of different types of coffee and concentrations of SCOBY is thought to have a significant effect on the chemical and microbiological characteristics of kombucha cascara.

CHAPTER 2
LITERATURE REVIEW

2.1. Coffee

Coffee (*Coffea sp*.) is a type of dicotyledonous plant and has a taproot. The plants grow upright and have branches. They can reach up to 12 meters. There are many types of coffee in the world but the types that are usually cultivated by farmers are arabica, robusta and liberica. Coffee consists of four parts, namely the exocarp, the mesocarp, the parchment, and the endosperm.

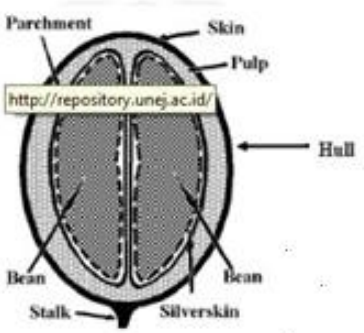


Figure 2.1 Coffee Structure (Widyotomo, 2012)

According to Nuraini *et al.* (2015), coffee fruit consists of fruit skin (45%), coffee beans (40%), mucilage (10%), and seed coat (5%). The mucus portion contains bound water by 85% and colloidal material that does not contain water and is hydrophilic by 15%. Coffee beans are protected by fruit skin, fruit flesh, mucus layer, horn skin and epidermis which can be seen in Figure 2.1.

2.1.1. Characteristics and Content of Arabica

Figure 2.2. Arabica (Fitrisa, 2020)

Arabica is the best coffee in taste and quality compared to other types of coffee. Arabica (*Coffea arabica*) is the first type of coffee to be cultivated. The characteristics of Arabica coffee are short beans and wavy dark green leaves. Arabica coffee beans are quite large with a weight of 18-22 g per 100 beans. The color of the seeds is slightly brown and seeds that are well processed will have a slightly bluish and greenish color. Good quality beans with a distinctive strong Arabica coffee taste and a slightly sour taste with a caffeine content of 1-1.3%. Based on Anggara and Marini (2011), arabica coffee has the following characteristics, (1) has a fragrant aroma similar to a combination of floral and fruit, (2) the texture becomes thicker when absorbed, (3) tastes slightly bitter, (4) has a bitter taste. The sour taste that robusta does not have, and (5) the taste is soft.

2.1.2. Characteristics and Content of Robusta Coffee

Robusta development was initiated to develop coffee that is more resistant to leaf rust disease. Robusta (*C. canephora var. Robusta*) is more resistant to leaf rust disease than arabica coffee and liberica coffee. Until now, Indonesian coffee plantations are dominated by the type of robusta coffee and have been mass-produced in Sumatra and Java.

Figure 2.3. Robusta (Risnandar and Fahmi, 2018)

Robusta has a wider crown and larger leaves. The beans are slightly rounded, thick curves and the diameter from top to bottom is almost the same. Robusta coffee leaves grow opposite the stems, branches and twigs (Najiyatih and Danarti, 2012). This coffee has a taste similar to chocolate, more bitter than arabica, slightly sour, and has a characteristic sweet smell. Among all types of coffee, robusta has the best resistance to the leaf rust disease or *Hemileia vastatrix* (HV). More than 90% of coffee plantations in Indonesia are robusta plantations (Prawosto et al., 2010).

2.2. Cascara

The intensity of coffee husk waste generated from the coffee processing process encourages the development of the derivative products of coffee husk, one of which is cascara. Linguistically, cascara is included in the Spanish vocabulary, which means skin. According to Pabari (2014), cascara is dried coffee skin. Meanwhile, according to Savitri (2021), cascara is an herb made from coffee skin, shaped like a cherry and is often used as a coffee or tea drink. Cascara has a sweet taste and a distinctive aroma like herbal tea with aromas such as mangoes, cherries, rose petals and even tamarind (Carpenter, 2015). Cascara brew has a fruity flavor with a blend of strawberry and raisin aromas (Nurhayati et al., 2020) as well as rose, cherry, mango and tobacco blends (Muzaifa *et al*., 2019).

Figure 2.4. Cascara (Hakim, 2020)

Cascara is produced from drying coffee skins in the sun by utilizing temperatures around 30-35°C for 2-3 weeks. Based on the research of Nafisah et al. (2018), the highest total phenol content in cascara resulted from drying treatment-using sunlight. This is because the low temperature can maintain the polyphenol components in the cascara. Another drying method that can be used is drying using artificial heat from the oven. Oven drying is considered to be more beneficial for product quality because the decrease in water content occurs in large quantities in a short time (Subyekti, 2012).

The process of making tea from coffee husks consists of several stages, namely sorting, washing coffee cherries, peeling and drying coffee pods (Galanakis, 2017). Cascara development is not optimal even though it has so many benefits. According to Bondesson (2015), per 100 grams of cascara contains polyphenol components in the form of catechins and epicatechins as

contained in tea. Cascara also contains components similar to coffee, namely chlorogenic acid (2.5 g) and very low levels of caffeine (1-1.3 g). Meanwhile, according to Heeger et al. (2017) and Nurhayati et al. (2020), the caffeine content of cascara is 226 mg caffeine/L. These components are known as antioxidants or anti-free radicals that are able to delay cell damage so that they can be a refreshing drink as well as an alternative in the therapy of high blood pressure, heart disease and cancer (Al-Yousef *et al*., 2017). Cascara has a potential as a source of antioxidants because it contains several antioxidants such as gallic acid, protocatechuic acid, chlorogenic acid and rutin. The antioxidant content of cascara can be seen in Table 2.2.

Table 2.1. The antioxidant content of cascara

No.	Content	Amount
1.	Cafein (mg/L)	226.4 ± 1.2
2.	Fenol (mg/L)	
	2.1 Gallic acid	4.3 ± 0.5
	2.2 Protocatechuic acid	85.0 ± 0.5
	2.3 Chlorogenic acid	69.6 ± 0.4
	2.4 Routine	6.1 ± 0.0
3.	Antioxidant Activity	
	3.1 ORAC (mmol/TE/L)	8.86 ± 0.186
	3.2 ABTS (mmol/TE/L)	3.02 ± 0.006
	3.3 TPC (mg of GAE/L)	283 ± 12.0

Source: Heeger *et al*. (2016)

The oxygen radical absorbance capacity or ORAC value of the cascara is 22,070 mol TE/100 g or 4 times higher than the ORAC value of blueberries and equivalent to the value of chili powder and goji fruit (Nurhayati et al., 2020). The inhibitors of the -glucosidase and -amylase enzymes contained in cascara are good for consumption by diabetics (Subeki et al., 2019).

The brown color of cascara is caused by changes in the color of the coffee husk pigment during drying. Drying of coffee husks into cascara products is generally done using sunlight (Yuliandri, 2016). However, it is susceptible to contamination so that hygiene and sanitation are not maintained. Esquivel and Jimenez (2012) stated that coffee husk contains anthocyanin pigments which contribute to the red color of coffee husk. According to Urbahillah (2018) the drying process will cause a decrease in the color stability of anthocyanins. The decrease in anthocyanin stability is caused by the degradation of anthocyanins from aglycone form to chalcone which will then form brown colored -diketone.

2.3. Kombucha

Kombucha comes from two words namely *kombu* and *cha*. Kombu is the name of a healer who came from Korea and lived in the 5th century AD. Cha is taken from Chinese that means tea. Kombucha is a fermented tea drink with added sugar and fermented with a kombucha starter, which is a symbiotic bacterial symbiosis of *Acetobacter xylinum* and several types of yeast, including *Saccharomyces cereviseae* where the fermentation process is carried out for 1-2 weeks (Sun *et al*., 2015). Meanwhile, according to Wistiana and Zubaidah (2015), kombucha tea is a traditional fermented beverage product from a solution of tea and sugar using a kombucha starter (*Acetobacter xylinum* and several types of yeast) where kombucha has health effects including antioxidant, antibacterial, able to increase immunity and so on. Based on Yanti *et al.* (2020), kombucha is a fermented drink from tea liquid containing sugar by a consortium of microorganisms from the acetic acid bacteria group, namely *Acetobacter xylinum* and the yeast *Saccharomyces sp*. The dominant microorganisms found in kombucha include *Acetobacter, Gluconobacter,* and the yeast groups *Saccharomyces, Schizosaccharomyces* and *Zygosaccharomyces.*

Figure 2.5. Kombucha (Lararenjana, 2020)

The yeast group will use the sugar in the tea solution as a substrate and convert it to alcohol. The alcohol produced will be consumed by bacteria to produce organic acid compounds. The dominant acid compound in kombucha is acetic acid. The major components that will be produced during the fermentation process are acetic acid, ethanol, and glucuronic acid while the minor components produced are lactic acid, phenolic acid, B vitamins and enzymes (Suhardini *et al.* 2016).

2.3.1. Kombucha Chemical Ingredients

Kombucha is known as a drink that can overcome various health problems including diabetes, obesity, rheumatism, arthritis, and so on (Ningtyas, 2015). Kombucha culture is able to convert sugar and convert it into substances that are beneficial to the body such as glucuronic acid, lactic acid, vitamins, amino acids, antibiotics and other substances. The chemical composition of kombucha tea is shown in Table 2.3.

Table 2.2. Chemical content in 120 mL of kombucha

Contents	Amount
Calori (Cal)	40.00
Total Carbohydrate (g)	8.00
Protein (g)	0.00
Total Fat (g)	0.00
Sugar (g)	8.00
Vitamin C (mg)	0.12
Sodium (g)	0.00
Folat Acid (mg)	0.64
Riboflavin (mg)	1.16

Source: Nurul (2010)

2.4. SCOBY (*Symbiotic Culture of Bacteria and Yeast*)

SCOBY or Symbiotic Culture of Bacteria and Yeast is a secondary metabolite obtained from kombucha fermentation that forms a cellulose structure (Rusdiana, 2017).

Figure 2.6. SCOBY (Putri, 2020)

Kombucha culture consists of symbiotic microorganisms. Based on the results of research that have been known, the microorganisms found in the culture used are acetic acid bacteria and yeast. The results of the gram staining observations showed that the morphology of the microorganism cells was red and coccus-shaped, so it was concluded that the bacteria were classified as gram-negative bacteria, namely

Acetobacter sp. In the results of the microscope, it can also be seen if the morphology of the yeast cells found in kombucha is oval or eclipsed. Yeasts identified in SCOBY are Saccharomyces, Zygosaccharomyces, and Brettamyces (Efivani, 2017).

CHAPTER 3
RESEARCH METHODOLOGY

3.1. Place and Time

This research was carried out at the Agricultural Product Chemistry Laboratory, General Microbiology Laboratory and Agricultural Product Processing Laboratory, Agricultural Products Technology Study Program, Agricultural Technology Department, Faculty of Agriculture, Sriwijaya University, Indralaya. The research started in July to August 2021.

3.2. Tools and Materials

The tools used in this study were: 1) glassware for analysis, 2) autoclave (Hirayama, Japan), 3) basin, 4) glass bottle, 5) incubator (Memmert, Germany), 6) paper, 7) label, 8) refrigerator, 9) analytical balance, 10) test tube clamp, 11) 80 mesh filter, 12) pulper, 13) refractometer (ATAGO Master), 14) spectrophotometer (Jenway, UK), 15) glass jar with faucet, 16) cimarec thermo scientific stirring hotplates (Cimarec, US), 17) vortex (Maxi II, type 3760, Germany), and 18) aluminum crucible.

The materials used in this study were: 1) water, 2) aquadest, 3) chemicals for analysis, 4) Pagaralam arabica coffee husk, 5) Pagaralam robusta coffee husk, 6) SCOBY (symbiotic culture of bacteria and yeast) purchased through the e-commerce platform shopee, and 7) sucrose.

3.3. Research Method

This study used a Factorial Completely Randomized Design (RALF) with two treatment factors, namely (A) type of coffee consisting of 2 treatment levels and (B) SCOBY concentration consisting of 3 treatment levels, in order to obtain 6 treatment combinations. Each treatment was replicated 3 times to obtain 18 experimental units. The treatment factors are as follows:

1. Coffee Type (A)

A_1 = Arabica

A_2 = Robusta

2. Concentration SCOBY

B_1 = Concentration SCOBY 5% (b/v)

B_2 = Concentration SCOBY 10% (b/v)

B_3 = Concentration SCOBY 15% (b/v)

Description:

b/v = the weight of addition of ingredients per total volume of kombucha

3.4. Data Analysis

The data obtained were processed using analysis of variance or ANOVA. Treatments that had a significant effect were further tested using the Honest Significant Difference (HSD) test at 5% level.

3.5. Statistical analysis

The data obtained were processed using statistical analysis. Data processing was carried out quantitatively using parametric statistical analysis data processing techniques.

According to Gomez and Gomez (1995), experimental data were analyzed using a Factorial Completely Randomized Design (RALF) with two treatment factors which can be stated as follows:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

Description:

Y_{ijk} = observation value

μ = average value

α_i = effect of coffee type

β_j = effect of SCOBY concentration

$(\alpha\beta)_{ijk}$ = the interaction effect of the type of coffee and the concentration of SCOBY

ε_{ijk} = trial error (galat)

The measurement results were processed through parametric statistical analysis. The analysis of variance in statistics can be seen in Table 3.1.

Table 3.1. List of Factorial Completely Randomized Design Variance Analysis (FCRD)

Source of Variance (SD)	Degree of Freedom (df)	Sum of Square (SS)	Sum of Square of Median (SSM)	F_{count}	F_{Table} 5%
Treatment	V_1=(m.n)-1	JKP	JKP/V_1	KTP/KTG	(V_1,V_2)
Factor A	V_2=(m-1)	JKA	JKA/V_2	KTA/KTG	(V_2,V_5)
Factor B	V_3=(n-1)	JKB	JKB/V_3	KTB/KTG	(V_3,V_5)
Interaction AB	V_4=(m-1)(n-1)	JKAB	JKAB/V_4	KTAB/KTG	(V_4,V_5)
Galat	V_5=V6-V1	JKG	JKG/V_5		
Total	V_6=(m.n.r)-1	JKTotal	JKTotal/V_6		

Source: Gomez dan Gomez (1995)

Description:

m = number of treatments A

n = number of tratments B

r = number of repetation

The significance of the variance analysis was carried out by comparing the F_{Table} in the 5% test. The basis of comparison was based on Gomez and Gomez (1995) as follows:

If the F_{count} obtained was more than or equal to the FTable 5%, it was declared to have no significant effect and was marked with *.

If the F_{count} obtained was less than or equal to the FTable 5%, it was declared to have no significant effect and was marked with ns.

If the results of the analysis of variance showed that F_{count} is greater than FTable, then proceeded with the HSD test (Honest Significant Difference) to find out the mean difference in each experiment. The formula used for the HSD test was as follows:

$HSD\alpha = Q\ \alpha_{(p,v)} . S\bar{y}\alpha$

$HSD\beta = Q\ \beta_{(p,v)} . S\bar{y}\beta$

$HSD\alpha\beta = Q\ \alpha\beta_{(p,v)} . S\bar{y}\alpha\beta$

$$S\bar{y}\ \alpha = \sqrt{\frac{KTG}{3 \times r}}$$

$$S\bar{y}\ \beta = \sqrt{\frac{KTG}{2 \times r}}$$

$S\bar{y}\ \alpha\beta = \sqrt{\frac{KTG}{r}}$

Description:

αβ = Value of HSD at 5%

p = number of treatment tested

v = error-free degree

Sȳ = common mean standard error

CSE = center square of error

r = repetation

To determine the level of accuracy according to Gomez and Gomez (1995), the Coefficient of Variance (CD) test was used. If the value of the Coefficient of Variance (CD) obtained was less than 15%, it meant that this research had good accuracy. The coefficient of variance was calculated by the following formula:

$$KK(\%) = \frac{\sqrt{KTG}}{Y} x\ 100$$

Description:

CV = coefficient of variance

CSE = center square of error

Y = the average value of all experimental data

3.6. Procedures

This research consisted of several stages of research, namely the stage of making kombucha cascara and the stage of analysis. The way the kombucha cascara worked was based on the modified Urbahillah (2018) method, which was as follows:

1. Sucrose 10% (w/v) was boiled with 3 L of water until it boiled, then 1% cascara (w/v) was added while stirring for 5 minutes.
2. The cascara solution was filtered using an 80 mesh filter and then cooled to 40°C.

3. Then the cascara solution was put into a glass jar with a faucet and inoculated with 5%, 10% and 10% kombucha culture (SCOBY). 15% (w/v).
4. After that, it was done with aerobic fermentation indoors (temperature range from 20-25°C) and not exposed to direct sunlight for 8 days (Nurhayati, 2020).
5. Finally, the fermented cascara solution was separated from the kombucha culture (SCOBY) and packaged in glass bottles and then analyzed.

3.7. Parameters

3.7.1. Total Polyphenol Test

The measurement of the total polyphenol content of kombucha cascara using the *Follin-Ciocalteu* method according to the method developed by Slinkard and Singleton (1977) was as follows:

1. 0.1 mL of kombucha sample was put into a test tube and 4.9 mL of distilled water was added.
2. Follin-Ciocalteu reagent was added as much as 0.5 mL into a test tube and vortexed and then allowed to stand for 5 minutes.
3. A total of 1 mL of 7% Na2CO3 solution is added and vortexed so that the solution is homogeneous.
4. The test tube was left in a dark place for 60 minutes.
5. Value of absorbance using a spectrophotometer at a wavelength of 765 nm.
6. Measurement of the blank solution was carried out in the same way but the sample was replaced using distilled water in the same amount.
7. Total polyphenols were calculated using standard curves prepared from gallic acid at various concentrations.
8. Total phenol of the sample was expressed as mg GAE/mL with GAE = gallic acid equivalent.

3.7.2. Total Titratable Acidity (TTA) Test

Total Titratable Acidity (TTA) test was carried out using the acid-alkali titration method based on Cahyaningtyas (2018). The steps taken to measure the total titrated acid (%) were as follows:

NaOH . Standardization

1. A standard solution of 0.1 M acetic acid was prepared by taking 0.5 mL of glacial acetic acid solution into a 100 mL volumetric flask, then adding distilled water to the boundary line.
2. A total of 100 mL of acetic acid was put into a 250 mL Erlenmeyer and added with 1% phenolphthalein (pp) indicator.
3. The solution is titrated using NaOH solution until a pink color is formed which lasts for 15 seconds.
4. The normality of NaOH was calculated using the formula:

$$Normality\ \text{NaOH (N)} = \frac{Mass\ \ CH_3COOH\ (g) x\ n\ CH_3COOH\ x\ 1000}{Mr\ CH_3COOH\ x\ V\ NaOH\ (mL)}$$

Measurement of Total Acid Content Titrated Kombucha Cascara

1. A total of 10 mL of kombucha cascara was taken and put into a 100 mL volumetric flask.
2. It was added with distilled water up to the mark and then homogenized.
3. Took 50 mL and put it in an Erlenmeyer then added 3 drops of *phenolphthalein* indicator (pp).
4. Titrated with 0.1 N NaOH solution until a pink color was formed. Read the scale when the red color was first formed and lasted for 15 seconds.
5. The total acetic acid content (%) was measured using the formula:

$$Total\ Acetic\ Acid\ \ (\%) = \frac{V\ titran\ x\ N\ titran\ x\ P\ x\ BM\ CH_3COOH}{\text{V sample (mL) x 1000}} x\ 100\%$$

Description:

V = volume of NaOH solution as titrant (mL)

N = normality of NaOH

P = dilution amount

BM = acetic acid molecular weight (60)

V sampel = kombucha sample volume (mL)

3.7.3. Total Dissolved Solids

The measurement of total dissolved solids was based on the AOAC (1999) with the following steps.

1. The refractometer was prepared.
2. The prism glass cover was opened and then 1-2 drops of sample were placed on it.
3. The prism glass cover was closed slowly.
4. The refractometer was directed at a bright light and then the scale reading was seen through the binoculars.
5. If the scale was blurred, rotate the binocular hole until the scale reading was clear.
6. Total dissolved solids were expressed in °Brix.

3.7.4. pH Analysis

The measurement of pH was carried out using a pH meter based on the AOAC (1995) in the following way:

1. One 5 mL sample was prepared.
2. The pH meter was calibrated using pH 4 and 7 buffers.
3. The cathode was inserted into the sample and left until the number indicated on the digital measurement no longer changes.
4. For each time of the measurement, the cathode of the pH meter was rinsed with distilled water and then dried before being used again.

3.7.5. Total Plate Number Test

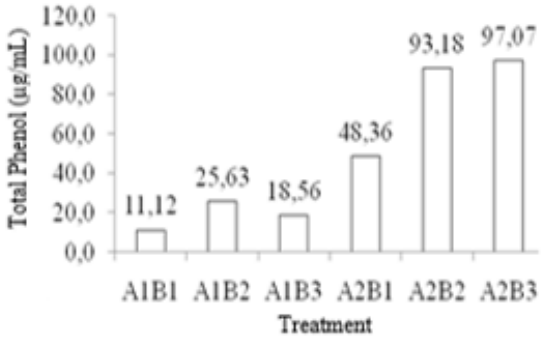
The calculation of the total plate number was carried out using Ulfa and Arfiana (2020) in the following way:

1. Kombucha which had been fermented for 8 days was taken as much as 1 mL and diluted to 10-3 dilution.
2. A total of 10 grams of nutrient was put into a Petri dish then the diluted kombucha sample was poured.
3. Petri dishes were incubated for 24 hours.
4. After 24 hours, the colonies formed were counted as the Total Plate Number (TPN).

CHAPTER 4
RESULTS AND DISCUSSION

4.1. Total Phenol

The measurement of total phenol was carried out to determine the amount of phenol content of a solution in g/mL. The measurement of total phenol in this study was done because cascara was known to have a high phenol content (Heeger et al., 2016). Research by Ayu *et al*. (2013) showed that the total phenol of local Balinese black tea kombucha fermented for 8 days was 43.28 g/mL. Meanwhile, Nurhayati *et al.* (2020) in her research stated that the total phenol value of kombucha cascara ranged from 6.3 to 36.9 mg/mL. The results showed that the average total phenol value of kombucha cascara ranged from 11.12 g/mL to 97.07 g/mL. The highest total phenol content was obtained by treatment A2B3 (kombucha cascara robusta fermented with 15% SCOBY concentration) of 97.07 g/mL. Meanwhile, the lowest total phenol content was obtained by A1B1 treatment (fermented kombucha cascara arabica with 5% SCOBY concentration) of 11.12 g/mL. The results of the measurement of total phenol in kombucha cascara with different types of coffee husks used at various concentrations of SCOBY can be seen in Figure 4.1



Description:

A_1	= Arabica husk	B_1	= Concentration SCOBY 5%
A_2	= Robusta husk	B_2	= Concentration SCOBY 10%
		B_3	= Concentration SCOBY 15%

Figure 4.1. The average total phenol value (µg/mL) of kombucha cascara

The results of the analysis of variance showed that the treatment factor A (coffee skin type) had a significant effect on the total phenol value of kombucha cascara produced, but factor B (SCOBY concentration) and the interaction of the two treatment factors had no significant effect on the total phenol in kombucha. Phenol levels in raw materials, namely arabica and robusta husks affected the total phenol content in kombucha. In general, the phenols found in the coffee husk were flavan-3-ol, hydroxynamic acid, flavonols, anthocyanidins, catechins, epicatechins, rutin, tannins and ferulic acid (Esquivel and Jimenez, 2012). Meanwhile, based on the analysis of raw materials that had been carried out, Arabica husk tea contained phenol levels of 32.70 g/mL. This amount was smaller than the phenol content in robusta coffee skin tea, which was 33.14 g/mL. This was in line with the measurement results of the total phenol of kombucha cascara where the total phenol of kombucha cascara robusta was greater than the total phenol of kombucha cascara arabica. The results of the 5% HSD test, the effect of the coffee husk type treatment factor on the total phenol of kombucha cascara can be seen in Table 4.1.

Table 4.1. The result of further test HSD 5% the effect of coffee husk type on the total phenol of kombucha cascara

Coffee husk type	Total phenol mean (GAE/mL)	HSD 5% = 26.49
A_1 (Arabica husk)	55.31	a
A_2 (Robusta husk)	238.61	b

Description : numbers followed by the same letter in the same column shows that the treatment was not significantly different

The results of the 5% HSD test (Table 4.1) showed that the total phenol of kombucha cascara robusta was significantly different from the total phenol of kombucha cascara arabica. Kombucha cascara robusta has a higher phenol content than cascara arabica at all SCOBY concentrations. The results obtained were in line with the results of Ariadi's (2013) research in Solichah (2019) which reported that the phenol content of cascara robusta was higher than cascara arabica in every condition of the brewing process. Murlida et al. (2021) also stated that among the three types of cascara used in their research, cascara robusta tea had the highest phenol content, followed by cascara liberika tea and cascara arabica tea had the

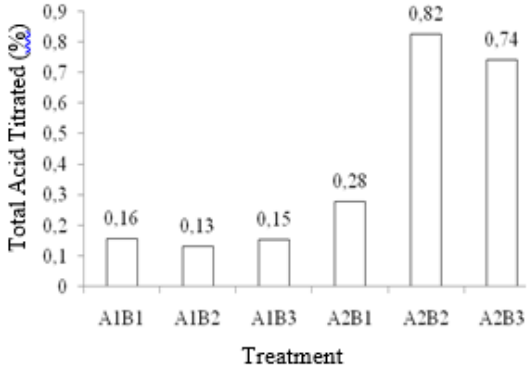
lowest phenol content. Based on Farah (2012), indirectly, total phenol interpreted chlorogenic acid levels as the main phenolic compounds contained in cascara. The higher the total phenol, the higher the chlorogenic acid content.

Although the concentration of SCOBY did not have a significant effect, according to Suhardini and Zubaidah (2016), the increase in phenolic compounds in tea of various types of leaves that were processed into kombucha was caused by the activity of various groups of bacteria and yeasts that can metabolize to produce flavonoid compounds through enzymatic reactions. Sukmawati *et al.* (2012) in Hunandar (2016) also stated the same thing that on the 10th day of kombucha fermentation there was an increase in phenol levels caused by biotransformation of several phenolic compounds due to enzymes released and the release of catechins from acid-sensitive microorganisms. Bhattacharya *et al.* (2011) in Nurhayati *et al.* (2020) stated that the enzymatic activity produced by SCOBY degraded matrix components to form phenolic compounds.

Volmer *et al.* (2017) in Nurhayati *et al.* (2020) also explained that the increase in phenol levels occurred due to the degradation of chlorogenic acid by microorganisms into caffeic acid. Caffeic acid is a flavonoid phenolic compound. Caffeic acid was then broken down into cinnamic acid. Meanwhile, according to Ayurtri and Kusnadi (2017) Saccharomyces cerevisiae was able to produce phenol reductase enzymes and decarboxylate cinnamic acid. Cinnamic acids such as pyrulic acid and p-caumaric acid are decarboxylated to form phenolic compounds such as 4-vinyl guaiacol (4-VG) and 4-vinyl phenol (4-VP).

4.2. Total Acid Titrated

Total acid was one of the important parameters to be observed during the fermentation process. This was because the fermentation process produced metabolites in the form of organic acids (Afriani, 2010). Based on the measurement of total titrated acid, cascara arabica tea contained 0.042% titrated acid and cascara robusta tea contained 0.018% titrated acid. After being processed into kombucha, the total titrated acid increased significantly. This increase occurred because during the fermentation process, yeast and bacteria remodel sucrose into organic acids (Urbahillah, 2018). The total titrated acid of kombucha cascara ranged from 0.132% to 0.824%. The results of the measurement of total acid titrated in kombucha cascara with different types of coffee husks and SCOBY concentrations can be seen in Figure 4.2.



Description

A_1	= arabika husk	B_1	= Concentration SCOBY 5%
A_2	= robusta husk	B_2	= Concentration SCOBY 10%
		B_3	= Concentration SCOBY 15%

Figure 4.2. The average total titrated acid value (%) kombucha cascara

The total titrated acid of kombucha cascara arabica obtained ranged from 0.132-0.156%. This value did not meet the quality requirements of kombucha according to KBI (Kombucha Brewers International) which stated that the total titrated acid of kombucha ranged from 0.27-2.03%. Meanwhile, the total titrated acid of kombucha cascara robusta which ranged from 0.28-0.82% was in accordance with the quality requirements set by KBI.

The results of the analysis of the variance of total titrated acid in kombucha cascara showed that treatment factor A (coffee husk type), treatment factor B (SCOBY concentration) and the interaction of the two factors had a significant effect on the total titrated acid in kombucha cascara. The results of the 5% HSD test showed the effect of the treatment factors of different types of coffee skins on the total titrated acid of kombucha cascara can be seen in Table 4.2.

Table 4.2 Results of further test HSD 5% the effect of different types of coffee treatment factors on total acid titrated kombucha cascara

Coffee husk type	Total Acid Titrated mean (%)	HSD 5% = 0.0295
A_1 (Arabica husk)	0.44	a
A_2 (Robusta husk)	1.84	b

Description : numbers followed by the same letter in the same column shows that the treatment was not significantly different

The results of the 5% HSD test (Table 4.2) showed that the total titrated acid of kombucha cascara robusta was significantly different from that of kombucha cascara arabica. The total acid in cascara processed products was influenced by the chlorogenic acid content contained in it. Nurhayati *et al*. (2013) in her research stated that chlorogenic acid was the dominant phenolic compound in cascara which was 69.6 mg/L. The higher total titrated acid in kombucha cascara robusta indicated that robusta coffee husk contained higher chlorogenic acid than Arabica coffee husk. Based on Farah and Donangelo (2006), the chlorogenic acid content of arabica, liberica and robusta coffee was 6.88%, 6.97% and 7.17%, respectively.

This was also directly proportional to the total phenol value obtained in this study, where the total phenolic content of kombucha cascara robusta was higher than the total phenol of kombucha cascara arabica which indicated that the content of chlorogenic acid phenolic compounds in robusta coffee husks was higher than arabica coffee husks.

The higher the concentration of SCOBY in the kombucha cascara fermentation, the higher the total titrated acid as well. The results of the 5% HSD further test the effect of treatment factors increasing the concentration of SCOBY on the total titrated acid of kombucha cascara can be seen in Table 4.3.

Table 4.3. Results of further test HSD 5% the effect of treatment factors increasing the concentration of SCOBY on the total titrated acid of kombucha cascara

SCOBY Concentration	Total Acid Titrated mean (%)	HSD 5% = 0.0443
B_1 (SCOBY Concentration 5%)	0.65	a
B_2 (SCOBY Concentration 15%)	1.34	b
B_3 (SCOBY Concentration 10%)	1.43	c

Description: the numbers followed by the same letter in the same column indicate that the treatment was not significantly different

The results of the 5% HSD test (Table 4.3) showed that fermented kombucha with 10% SCOBY concentration was significantly different from kombucha fermented with 5% and 15% SCOBY concentrations. The total value of titrated acid in fermented kombucha with a concentration of 10% SCOBY resulted in the highest total titrated acid. According to Cahyani (2015), this was because the concentration of SCOBY would affect the production of acetic acid in kombucha where the SCOBY concentration of 10% and the use of 10% sucrose were the

optimum concentrations in making kombucha and produced the highest acetic acid. The concentration of SCOBY 10% was proportional to the amount of substrate so that the substrate can be used for growth, cell multiplication and optimal production of organic acids. Ardheniati *et al.* (2009) also explained that the decrease in acetic acid levels was influenced by the decreasing supply of sugar, so that acetic acid bacteria oxidized acetic acid in obtaining energy for growth. Urbahillah (2018) in his research also explained that the concentration of acetic acid in kombucha only increased to a certain extent. This was because the sugar content had been exhausted and acetic acid was further utilized by *Acetobacter xylinum.*

The interaction between coffee husk and variations in SCOBY concentration showed a significant effect on the total titrated acid of kombucha cascara. The results of the 5% HSD further test showed the interaction effect of the two treatment factors on the total titrated acid of kombucha can be seen in Table 4.4.

Table 4.4. Results of further test HSD 5% the effect of the interaction of the two treatment factors on the total titrated acid of kombucha cascara

Coffee husk and SCOBY Concentration	total titrated acid mean (5%)	HSD 5% = 0.0322
A_1B_2 (arabika, SCOBY 10%)	0.13	a
A_1B_3 (arabika, SCOBY 15%)	0.15	a
A_1B_1 (arabika, SCOBY 5%)	0.16	a
A_2B_1 (robusta, SCOBY 5%)	0.28	b
A_2B_3 (robusta, SCOBY 15%)	0.74	c
A_2B_2 (robusta, SCOBY 10%)	0.82	d

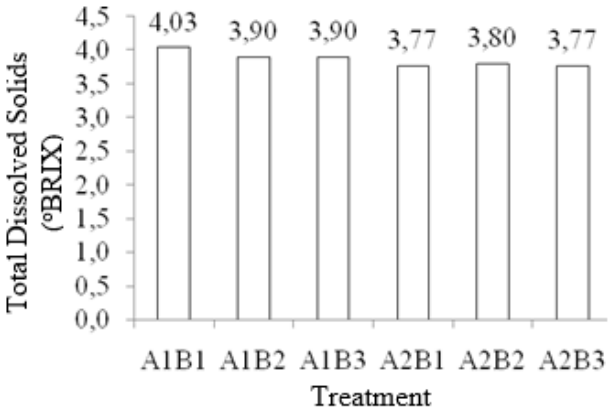
Description: the numbers followed by the same letter in the same column indicate that the treatment was not significantly different

The results of the 5% HSD test (Table 4.4) showed that the A2B2 (kombucha cascara robusta fermented with 10% SCOBY concentration) treatment was significantly different from the other treatments. Kombucha cascara robusta with 10% SCOBY concentration produced the highest total titrated acid of 0.824%. Robusta coffee husk produced a higher total titrated acid than Arabica coffee husk. Meanwhile, the 10% SCOBY concentration and the addition of 10% or 1:1 sucrose were the optimum concentrations to produce the highest acetic acid in kombucha tea fermentation (Cahyani, 2015). Verawati (2019) in her research stated that the yeast and bacteria found in kombucha got energy by degrading sugar bonds into ATP. So that if the available substrate was less than the number of

microorganisms, then the results of the overhaul used for cell multiplication would be more and more while the production of organic acids will be less. In contrast, if the available substrate was more than the number of microorganisms, the conversion of sugar into organic acids would not be optimal.

4.3. Total Dissolved Solids

The total dissolved solids shows the content of dissolved substances in the solution. Based on Muzaifa et al. (2019), total dissolved solids could interpret the sugar content in cascara steeping including kombucha cascara. Sintasari *et al*. (2014) stated that the total dissolved solids interpreted the remaining sugar content resulting from the overhaul of the fermentation process. Heeger *et al*. (2017) in his research reported that the water-soluble components in cascara included fructose, glucose, protein, and caffeine. Measurement results of total dissolved solids of kombucha cascara with different types coffee husk and SCOBY concentration can be seen in Figure 4.3.



Description

A_1	= Arabika husk	B_1	= Concentration SCOBY 5%
A_2	= Robusta husk	B_2	= Concentration SCOBY 10%
		B_3	= Concentration SCOBY 15%

Figure 4.3. Total dissolved solids (°Brix) value of average kombucha cascara

The total dissolved solids of kombucha cascara ranged from 3.77-4.03°Brix. The total soluble solids of cascara arabica tea which was initially 4.3°Brix decreased to 3.90-4.03°Brix after being processed into kombucha. The total dissolved solids of cascara robusta tea also decreased from 3.90°Brix to 3.77-3.80°Brix after being processed into kombucha.

The results of the analysis of the variance of total titrated acid in kombucha cascara showed that treatment factor A (coffee husk), treatment factor B (SCOBY concentration) and the interaction of the two factors had a significant effect on the total titrated acid in kombucha cascara. The results of the 5% HSD test showed the effect of the different types of coffee skin treatment factors on the total dissolved solids of kombucha cascara which can be seen in Table 4.5.

Table 4.5. Results of further test of 5% HSD the effect of different types of coffee treatment factors on the total dissolved solids of kombucha cascara

Coffee husk type	Total Acid Titrated mean (%)	HSD 5% = 0.0419
A_2 (Robusta husk)	11.3333	b
A_1 (Arabica husk)	11.8333	a

Description: the numbers followed by the same letter in the same column indicate that the treatment was not significantly different

The results of the HSD further test at 5% level (Table 4.5) showed that the total dissolved solids of kombucha cascara arabica was significantly different from that of kombucha cascara robusta. According to Heeger *et al*. (2017) in Nurhayati *et al.* (2020), the components contained in cascara consisted of water-soluble components, such as glucose, fructose, protein and caffeine. The higher total dissolved solids of kombucha cascara arabica indicates that cascara arabica contained more water soluble components. This was in accordance with research by Farah (2012) in Murlida *et al.* (2021) which stated that the sugar content of cascara arabica was higher than cascara robusta. Sugars, pigments, vitamins, organic acids and proteins were water soluble components (Ismawati et al., 2016).

In addition to internal factors, namely the content of cascara itself, one of the external factors that affect the total dissolved solids was the heating temperature. The use of a manual stove causes the specific temperature cannot be regulated. Pantastico (1986) in Muzaifa *et al.* (2019) stated that the higher the temperature, the faster the breaking of long chains of carbohydrate compounded into sugar. This resulted in a higher total dissolved solids.

The higher the concentration of SCOBY, the lower the total dissolved solids of kombucha cascara. The results of the 5% HSD further test showed the effect of

the treatment factor increasing the concentration of SCOBY on the total dissolved solids of kombucha cascara which can be seen in Table 4.6.

Table 4.6. Results of further test HSD 5% the effect of treatment factors increasing the concentration of SCOBY on the total dissolved solids of kombucha cascara

SCOBY Concentration	Total Dissolved Solids mean (°BRIX)	HSD 5% = 0.0628
B_3 (SCOBY Concentration 5%)	11.50	a
B_2 (SCOBY Concentration 15%)	11.55	a
B_3 (SCOBY Concentration 10%)	11.70	b

Description: the numbers followed by the same letter in the same column indicate that the treatment was not significantly different

The results of further HSD test at 5% level (Table 4.6) showed that the total soluble solids of fermented kombucha with 5% SCOBY concentration were significantly different from kombucha fermented with 10% and 15% SCOBY concentrations. Treatment B1 (5% SCOBY concentration) had the highest total dissolved solids content of 11.70°Brix. Based on Napitupulu *et al.* (2015), an increase in the number of microbes was accompanied by a decrease in the amount of substrate because it was consumed for metabolism which caused the value of total dissolved solids to decrease. Nurhayati *et al*. (2017) in her research described the activity and growth of microorganisms that degraded substrates such as sugar and the content of solutes in cascara and were used for microorganism metabolism so that the higher the number of microorganisms, the lower the dissolved solids.

The interaction between the type of coffee husk used and the concentration of SCOBY showed a significant effect on the total dissolved solids of kombucha cascara. The results of the 5% HSD further test the effect of the interaction of the two treatment factors on the total dissolved solids of kombucha cascara can be seen in Table 4.7.

Table 4.7. The result of further test HSD 5% interaction effect of coffee skin type and variation of SCOBY concentration on the total dissolved solids of kombucha cascara

Coffee husk and SCOBY Concentration	Total Dissolved Solids mean (°BRIX)	HSD 5% = 0,0457
A2B1 (robusta, SCOBY 5%)	3.77	a
A2B3 (robusta, SCOBY 15%)	3.77	a
A2B2 (robusta, SCOBY 10%)	3.80	a
A1B2 (arabika, SCOBY 10%)	3.90	b

A1B3 (arabika, SCOBY 15%)	3.90	b
A1B1 (arabika, SCOBY 5%)	4.03	c

Description: the numbers followed by the same letter in the same column indicate that the treatment was not significantly different

The results of the HSD test at 5% level (Table 4.7) showed that the A1B1 treatment (fermented kombucha cascara arabica with 5% SCOBY concentration) was significantly different from the other treatments. The A1B1 treatment, namely the interaction of A1 (arabic coffee) and B1 (5% SCOBY concentration) resulted in the highest total dissolved solids value of 4.03°Brix. Cascara arabica contains higher water soluble components than cascara robusta, resulting in a higher total dissolved solids. Meanwhile, the low concentration of SCOBY caused less substrate to be degraded so that the dissolved solids were higher (Nurhayati *et al.,* 2017). Combination treatment of fermented kombucha cascara arabica with 5% SCOBY concentration would produce the highest total dissolved solids. Meanwhile, the total dissolved solids of kombucha cascara robusta were not significantly different from one another at all SCOBY concentrations.

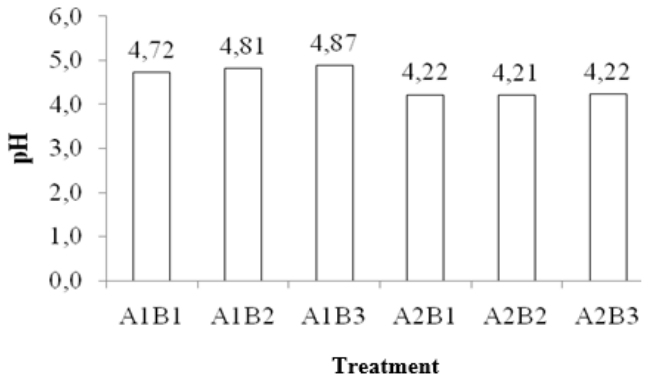
4.4. pH Analysis

The degree of acidity or pH was one of the important parameters to be observed in the fermentation process. The higher the acid content, the lower the pH of the product. The pH of kombucha cascara ranged from 4.20 to 4.87. Based on Afriani (2010), the fermentation process produced metabolites in the form of organic acids, causing a decrease in the pH of the fermentation product.

The pH standard for kombucha set by Kombucha Brewers International (2021) was 2.2-3.8. However, kombucha could still be consumed as long as the pH was below 4.6. The pH of kombucha cascara arabica ranged from 4.72-4.87 while the pH of kombucha cascara robusta ranged from 4.20-4.22. Based on these data, the pH of kombucha cascara arabica and robusta had passed the standard set by Kombucha Brewers International. However, kombucha cascara robusta was still below the acceptable limit for consumption.

Treatment factor A (coffee husk) used two different types of coffee skin to produce different pH. Based on the pH measurement of cascara tea that had been done, cascara arabica tea had a pH of 5.79, while cascara robusta tea had a higher pH of 6.33. After being processed into kombucha, the pH decreased. This decrease in pH was caused by the increasing total acid (Nurhayati *et al*., 2021). Ayuratri and Kusnadi (2017) explained that during fermentation, yeast would remodel sucrose

into glucose and fructose. Glucose was converted to gluconic acid by acetic acid bacteria. Meanwhile, most of the fructose was converted to acetic acid and a small amount of gluconic acid. It was the breakdown of sucrose into organic acids that increased acid levels and at the same time lowers pH. This statement was also supported by Urbahillah (2018) which explained that yeast and bacteria broke down sucrose into organic acids such as gluconic acid and acetic acid during the fermentation process, so that the total acid increased and the pH decreased. The results of measuring the pH of kombucha cascara with different treatment factors for coffee skin types can be seen in Figure 4.4.



Description

A_1	= Arabika husk	B_1	= Concentration SCOBY 5%
A_2	= Robusta husk	B_2	= Concentration SCOBY 10%
		B_3	= Concentration SCOBY 15%

Figure 4.4. Average pH value of kombucha cascara

Based on the calculation of the total titrated acid, kombucha cascara robusta had a higher acid content than kombucha cascara arabica, causing the pH of kombucha cascara robusta to be lower. This was in accordance with Adesokan *et al.* (2011) in Prastujati *et al.* (2018) which stated that the pH value of a fermented product was closely related to the acid content produced and had an inverse relationship with the total titrated acid where the higher the TAT value, the lower the pH value. The results of the 5% HSD test showed the effect of the coffee skin type treatment factor on the pH value of kombucha cascara which can be seen in Table 4.8.

Table 4.8. The results of the HSD further test 5% the effect of the coffee skin type treatment factor on the pH value of kombucha cascara

Coffee husk type	pH mean (%)	HSD 5% = 0.0922
A_2 (Robusta husk)	12.65	a
A_1 (Arabica husk)	14.40	b

Description: Numbers followed by the same letter in the same column indicate that the treatment was not significantly different

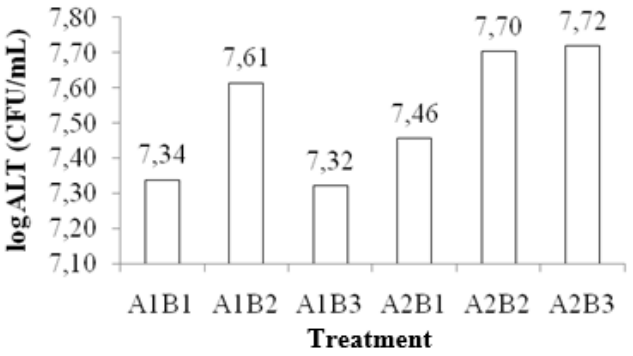
The results of the 5% HSD test (Table 4.8) showed that kombucha cascara arabica was significantly different from kombucha cascara robusta. This was related to the total titrated acid content of kombucha cascara robusta which was higher than the total titrated acid of kombucha cascara arabica in Table 4.2.

4.5. Total Plate Number

The total plate number or also called the total plate count was the number of mesophilic aerobic microorganisms per milliliter or per gram of sample determined using standard methods (BPOM RI, 2012). Villarreal-soto *et al.* (2018) reported that the number and types of microorganisms found in kombucha varied greatly. Jayabalan *et al.* (2014) added that the type and amount of microflora in kombucha varied greatly so that it cannot be determined absolutely what types were contained in it.

Based on the measurement of the total plate number, the total plate number of kombucha cascara ranged from log ALT 7.32 to 7.72 CFU/mL. Treatment factor A2B3 (kombucha cascara robusta fermented with 15% SCOBY concentration) had the highest ALT value of log 7.72 or 5.23 x 107 CFU/mL while the lowest ALT value was obtained by treatment A1B3 (kombucha cascara arabica fermented with SCOBY concentration). 15%) of log 7.32 or 2.09 x 107 CFU/mL.

Wistiana and Zubaidah (2015) in their research reported that the total kombucha microbes during fermentation ranged from 1.32 x 106 CFU/mL to 4.40×106 CFU/mL on day 8. Meanwhile, Sa'diyah and Lestari (2020) reported that the ALT value of kombucha tea fermented for 7 days was 79 x 103 CFU/mL. Fu et al. (2013) also stated that the average number of microorganisms in green tea kombucha was 2.49 × 107 CFU/mL.



Description

A_1	= Arabika husk	B_1	= Concentration SCOBY 5%
A_2	= Robusta husk	B_2	= Concentration SCOBY 10%
		B_3	= Concentration SCOBY 15%

Figure 4.5. The average total plate number (CFU/mL) of kombucha cascara

The results of the analysis of the variance of the total plate number of kombucha cascara showed that treatment factor A (types of arabica and robusta coffee skin), treatment factor B (SCOBY concentration 5%, 10%, 15%) and the interaction of the two factors gave no significant effect on the total plate number of kombucha. cascara. This could be influenced by the large variety of microflora in SCOBY used in kombucha fermentation.

Each microorganism and microflora had a different shape and size of the colony after incubation. In some samples it was possible to contain more microflora which made the colonies large so that the number of colonies that could be counted was low. Based on Hidayati (2019), there were at least 14 species of bacteria and 10 species of yeast in SCOBY. The bacteria in question included *Acetobacter xylinum as the dominant bacterium, Acetobacter aceti, Acetobacter pasteurianus, Gluconobacter, Brettanomyces, Bretanomyces bruxellensis, Brettanomyces intermedius, Candida, Candida fatama, Maycoderma, Mycotorula, Phichia, and Pichia membrana efacius*. While the yeasts in question included *Saccharomyces, Saccharomyces cerevisiae, Saccharomyces cerevisiae subp. Aceti, Scizoccharomyces, Torula, Torulaspora debruecki, Toruoplasis, Zygosaccharomyches, Zygosaccharomyces bailli, and Zigosaccharomyces rouziz.* According to Jamilah (2019), SCOBY contained at least some bacteria such as

Acetobacter xylinum, Kenicuminoides, Picobacterium varicolus, Acetobacter sp. ., and Pediococcus sp.

In kombucha cascara robusta, the higher SCOBY concentration resulted in a higher total plate number. Based on Wistiana and Zubaidah (2015), this increase was caused by the presence of soluble solids in kombucha cascara such as sugar, amino acids and caffeine. These substances were used by microorganisms as a source of energy and nutrients so that the number of microorganisms increased. Meanwhile, the decrease in the number of microorganisms from A1B2 (fermented kombucha cascara arabica with 10% SCOBY concentration) to A1B3 (fermented kombucha cascara arabica with 15% SCOBY concentration) was due to the fermentation process producing organic acids, alcohol and other substances that could inhibit growth. microorganisms. In addition, the decrease in the total plate number in the A1B3 treatment was also influenced by the content of phenolic compounds formed in the fermentation process where the phenol content had antimicrobial properties that could inhibit the growth of microorganisms (Simanjuntak and Mutiara, 2016).

CHAPTER 5
CONCLUSIONS AND SUGGESTIONS

5.1. Conclusions

The conclusions obtained from the results of this study were as follows:

1. Type of coffee husk, concentration of SCOBY and their interactions had a significant effect on total titrated acid and total dissolved solids of kombucha cascara.
2. The best treatment to get kombucha cascara with the best quality was A2B2 (robusta, 10% SCOBY) treatment with total phenol 93.18 GAE/mL, TAT 0.82%, TPT 3.80°Brix, pH 4.21, and ALT 5.04 CFU/mL.

5.2. Suggestions

The suggestion given for this research is that the sample preparation should be carried out directly by the researcher so that the preliminary treatment such as the length and temperature of drying of each type of coffee is uniform. Based on the results of the research that had been carried out, it is recommended to use the A2B2 treatment (type of robusta coffee, 10% SCOBY concentration) for product innovation of kombucha cascara. Further research that can be suggested is the identification of the type of acid present in kombucha, identification of microflora in kombucha, kombucha alcohol content test, the best fermentation time of kombucha cascara, and antimicrobial kombucha.

REFERENCES

Adiwati, P. dan K. Kusnadi. 2003. Kultur campuran dan faktor lingkungan mikroorganisme yang berperan dalam fermentasi teh cider. *Jurnal ITB Sains dan Teknologi*, 35(2), 147-162.

Adri, D. dan Hersoelistyorini, W. 2013. Aktivitas antioksidan dan sifat organoleptik teh daun sirsak (*Annona muricata* Linn.) Berdasarkan variasi lama pengeringan**.** *Jurnal Pangan dan Gizi*, 4(7).

Afifah, N. 2010. *Analisis kondisi dan potensi lama fermentasi medium kombucha (teh, kopi dan rosela) dalam menghambat pertumbuhan bakteri patogen (Vibrio cholerae dan Bacillus cereus).* Thesis. Malang: Universitas Islam Negeri Maulana Malik Ibrahim.

Afriani. 2010. Pengaruh penggunaan starter bakteri asam laktat Lactobacillus plantarum dan Lactobacillus fermentum terhadap total bakteri asam laktat, kadar asam dan nilai pH dadih susu sapi. *Jurnal Ilmu-Ilmu Peternakan*, 13(6): 279-285.

Anggara, A. dan Marini, S. 2011. *Kopi si hitam menguntungkan budi daya dan pemasaran.* Yogyakarta: Cahaya Atma Pustaka.

AOAC, 1995. *Official methods of analysis (14th ed)*. Washington DC: Association of Official Analytycal Chemistry Inc.

Apak, R., Cuclu, K., Demirata., N., Ozyurek, M., Celik, S.E., Bektasoglu, B. Berker K.I. dan Ozyurt, D. 2007. Comperative evaluation of various total antioxidant capacity assay applied to phenolic compounds with the cuprac assay. *Jurnal Molekul*, 12, 1496-1547.

Ardheniati, M., Andriani, M.A.M., Amanto, S. B. 2009. Kinetika fermentasi pada teh kombucha dengan variasi jenis teh berdasarkan pengolahannya. *Jurnal Biofarmasi*, 7(1), 48-55. ISSN: 1693-2242.

Ariadi., Harri, P. dan Windrawati, W. S. 2015. Compound Extraction of Coffee Fruit Cod: Study of Species and Maceration Duration of Coffee. *Berkala Ilmiah Pertanian*.

Ariva, A. N., Widyasanti, A. dan Nurjanah, S. 2020. Pengaruh suhu pengeringan terhadap mutu teh cascara dari kulit kopi arabika (*Coffea arabica*). *Jurnal Teknologi Dan Industri Pertanian Indonesia.*

Asosiasi Eksportir Kopi Indonesia. 2015. Industri kopi indonesia. http://www.aeki-aice. org/ page/ industri-kopi/ id. [Diakses pada 12 Maret 2021].

Ayuratri, M. K. dan J. Kusnadi. 2017. Aktivitas antibakteri kombucha jahe (*zingiber officinale*) (kajian varietas jahe dan konsentrasi madu). *Jurnal Pangan dan Agroindustri*, 5(3), 95-107.

Bhattacharya, S., Manna, P., Gachhui, R., Sil, P.C. 2011. Protective effect of kombucha tea against tertiary butyl hydroperoxide induced cytotoxity and cell death in murine hepatocytes. *Indian Journal of Experimental Biology*, 49(7), 511-524.

Bondesson, E. 2015. *A nutritional analysis on the by- product coffee husk and its potential utilization in food production*. Skripsi. Swedish University of Agricultural Sciences.

Bonilla-Hermosa, V. A., Duarte, W. F. dan Schwan, R. F. 2014. Utilization of coffee by-products obtained from semi-washed process for production of value-added compounds. *Bioresource Technology*, 166, 142–150. https://doi.org/10.1016/j.biortech.2014.05.031

Cahyani, Y. N. 2015. *Perbandingan kadar fenol total dan aktivitas antioksidan ekstrak metanol daun kopi robusta (Coffeea canephora) dan arabika (Coffeea arabica*). Skripsi. Fakultas Farmasi Universitas Jember.

Cahyaningtyas, Y. D. W. 2018. *Pengaruh lama fermentasi terhadap total asam tertitrasi (TAT) dan karakteristik fisik (uji organoleptik) pada teh kombucha serai (Cymbopogon citratus (dc.) Stapf)*. Skripsi. Yogyakarta: Universitas Sanata Dharma.

Carlsen, M.H., Halvorsen, B.L., Holte, K., Bohn, S.K., Dragland, S., Sampson, L., Willey, C., Senoo, H., Umezono, Y., Sanada, C., Barikmo, I., Berhe, N., Willett, W.C., Philips, K.M., Jacobs, D.R. dan Blomhoff, R. 2010. The total antioxidant content of more than 3100 foods, baverages, spices, herbs, and supplements sed worldwide. *Nutrition Journal*, 9 (3), 1-11.

Carpenter, M. 2015. Cascara tea: a tasty infusion made from coffee waste. www.npr.org/sections/thesalt/2015/12/01/456796760/*cascara*-tea-a-tastyinfusion-made-from-coffee-waste. [Diakses pada 12 Maret 2021].

Dewajanti, A. M. 2019. Peranan asam klorogenat tanaman kopi terhadap penurunan kadar asam urat dan beban oksidatif. *Jurnal Kedokteran Meditek*, 25(1), 46-51.

Direktorat Jenderal Perkebunan. 2015. *Statististik perkebunan indonesia: kopi 2014-2016*. Jakarta: Kementerian Pertanian.

Direktorat Jendral Perkebunan Republik Indonesia. 2017. *Buku statistik kopi*. Jakarta: Kementrian Pertanian.

Efivani, A.P. 2017. Pengaruh konsentrasi teh putih terhadap karateristik antioksidan serta potensi probiotik kombucha dengan pendekatan pemodelan matematik. *Skripsi*. Semarang: Universitas Katolik Soegijapranata.

Elfariyanti., Silviana, E. dan Santika, M. 2020. Analisis kandungan kafein pada kopi seduhan warung kopi di kota banda aceh. *Lantanida Journal*, 8(1), 1-95.

Esquivel, P. dan Jimenez, V. M. 2012. Functional properties of coffee and coffee by producctst. *Food Research International*, 46, 488 – 495.

Farah, A. 2012. *Coffee Constituent. In: Emerging Health Effect and Diseases Prevention ed Chu Yi-Fang.* John Willey and Sons Inc. Blackwell Publishing Ltd.

Fardiaz, S. 1993. *Mikrobiologi pangan I*. Jakarta: PT Gramedia Pustaka Utama.

Farmakologi UI. 2002. Farmakologi dan terapi edisi 4. Jakarta: Gaya Baru.

Febriyanto., Hanifa, N.I. dan Muliasari, H. 2021. Penetapan kadar fenolik total ekstrak kulit buah kopi robusta (*Coffea canephora* L.) di pulau lombok. *Jurnal Ilmu Kefarmasian*, 2(2).

Fitrisa, R. 2020. Harga kopi gayo anjlok, petani tagih jokowi: pemerintah tidak ada keseriusan. https://bekasi.pikiran-rakyat.com/nasional/pr-12837947/harga-kopi-gayo-anjlok-petani-tagih-jokowi-pemerintah-tidak-ada-keseriusan. [Diakses pada 13 Maret 2021].

Galanakis, C.M. 2017. *Handbook of coffee processing by-products: sustainable applications*. Unitied Kingdom: Academic Press.

Gomez, K. A. dan Gomez A.A., 1995. *Prosedur statistik untuk penelitian pertanian*. Edisi kedua. Jakarta: UI – Press.

Hakim, M. N. 2020. Pengolahan limbah kopi, ternyata berfaedah sekali: cascara. https://warstek.com/pengolahan-limbah-kopi-ternyata-berfaedah-sekali-cascara/. [Diakses pada 13 Maret 2021 pukul 16:50].

Heeger, A., Kosinska-Cagnazzo, A., Cantergiani, E. dan Andlauer, W. 2017. Bioactives of coffee cherry pulp and its ulitization for production of cascara beverage. *Food Chemistry*, 221, 969-975.

Hidayati, I. (2019). Efek lama fermentasi dan konsentrasi scoby (*symbiotic culture of bacteria and yeast*) terhadap kualitas fisiko kimia minuman kombucha jeruk nipis (*Citrus aurantifollia* s.). *Disertasi*. Malang: Universitas Muhammadiyah Malang.

Hunandar, Valentina Sintya. 2016. Penetapan daya antioksidan dan kadar total fenol kombucha dibandingkan teh hijau secara spektrofotometri. *Jurnal Ilmiah Mahasiswa Universitas Sriwijaya*, 5(2).

International Coffee Organization. 2019. *Total production by all exporting countries In thousand 60kg bags.* www.ico.org/prices/po-production.pdf. [Diakses pada 14 Maret 2021].

Ismawati, N., Nurwantoro dan Yoyok, B. P. 2016. Nilai pH, total padatan terlarut, dan sifat sensoris yoghurt dengan penambahan ekstrak bit (*Beta vulgaris* L.). *Jurnal Aplikasi Teknologi Pangan*.

Jamilah, V. 2019. *Pengaruh variasi konsentrasi starter terhadap kualitas teh kombucha*. Skripsi. Lampung: Universitas Islam Negeri Raden Intan.

Jayabalan, R., Malbaˇsa, R. V., Lonˇcar, E. S., Vitas, J. S. dan Muthuswamy Sathishkumar. 2014. A Review on Kombucha Tea—Microbiology,

Composition, Fermentation, Beneficial Effects, Toxicity, and Tea Fungus. *Comprehensive Reviewsin Food Science and Food Safety*.

Jayabalan, R., S. Marimuthu. dan K. Swaminathan. 2008. Changes in content of organic acids and tea polyphenols during kombucha tea fermentation. *Jurnal Food Chemistry,* 102, 392-398.

Jiménez-Zamora, A., Pastoriza, S. dan Rufián-Henares, J. A. 2015. Revalorization of coffee by-products. Prebiotic, antimicrobial and antioxidant properties. *LWT - Food Science and Technology*, 61(1), 12-18. https://doi.org/10.1016/j.lwt.2014.11.031.

Lararenjana, E. 2020. Tujuh manfaat kombucha bagi kesehatan, minum teh fermentasi sumber probiotik. https://www.merdeka.com/jatim/7-manfaat-kombucha-bagi-kesehatan-minuman-teh-fermentasi-sumber-probiotik-kln.html. [Diakses pada 14 Maret 2021 pukul 12:12].

Murlida, E., Noviasari, S., Nilda, C., Rohaya, S., Rahmi, F., dan Muzaifa, M. 2021. *Chemical characteristics of cascara tea from several varieties of coffee in Aceh Province*. The 2nd International Conference on Agriculture and Bio-industry IOP Conf. Series: Earth and Environmental Science. Banda Aceh: Universitas Syiah Kuala.

Muzaifa, M., Hasni, D., Arpi, N., Sulaiman, M. I. dan Limbong, M. S. 2019. Kajian pengaruh perlakuan pulp dan lama penyeduhan terhadap mutu kimia teh cascara. *Jurnal Teknologi Pertanian*, 23(2). ISSN 1410-1920, EISSN 2579-4019.

Nafisah, D. dan Widyaningsih, T. D. 2018. Kajian metode pengeringan dan rasio penyeduhan pada proses pembuatan teh cascara kopi arabika (*Coffea arabika* L.). *Jurnal Pangan dan Agroindustri*. 6(3), 37-47.

Najiyati, S. dan Danarti. 2012. *Kopi, budidaya dan pengolahan pasca panen*. Jakarta: PT. Penebar Swadaya.

Napitulu, MOW., Setyohadi, dan Lubis LM. 2015. Pengaruh variasi konsentrasi gula sukrosa dan lama fermentasi terhadap pembuatan kopi kombucha. *Jurnal Rekayasa Pangan dan Pert*, 3, 316-322.

Nguyen, K. N., N. T. N. Dong., P. H. Le. dan T. Nguyen. 2014. Evaluation of the glucoronic acid production and other biological activities of fermentes sweeten-black tea by kombucha layer and the co-culture with different lactobacillus sp. Strains. *IJMER*, 4(5), 2249-6645.

Nguyen, N. K., Nguyen, P.B., Nguyen, H.T., Le, P. H. 2015. Screening the optimal ratio of symbiosis between isolated yeast and acetic acid bacteria strain from traditional kombucha for high level production of glucoronic acid. *LWT-Food Sci Technol*, 64, 1149-1155. doi: 10.1016/j.lwt.2015.07.018.

Ningtyas, R. N. 2015. *Pengaruh lama fermentasi dan jumlah inokulum terhadap karakteristik kimia dan potensi antibakteri teh kombucha dari air rebusan*

jagung manis (zea mays saccharata sturt). Skripsi. Malang: Fakultas Sains dan Teknologi, Universitas Islam Negeri Maulana Malik Ibrahim.

Nuraini, N., Marlid, Y., Mirzah, M., Disafitri, R. dan Febrian R. 2015. Peningkatan kualitas limbah buah kopi dengan *Phanerochaete chrysosporium* sebagai pakan alternatif. *J Peternakan Indonesia*, 17, 143-150. DOI: 10.25077/jpi.17.2.143-150.2015.

Nurhayati., Yuwanti, S. dan Urbahillah, A. 2020. Karakteristik fisikokimia dan sensori kombucha cascara (kulit kopi ranum). *Jurnal Teknologi dan Industri Pangan*, 31(1), 39-49. ISSN 1979-7788.

Özpalas, B. dan Özer, E. A. 2017. Effects of caffeine on human health. *Nevşehir Billim ve Teknoloji Dergisi Cilt*, 6, 297-305.

Panggabean, E. 2011. *Buku pintar kopi*. Jakarta: Agro Media Pustaka.

Prastowo, B. E., Karmawati, Rubiyo, Siswanto, C. Indrawanto. dan Munarso. S. J. 2010. *Budidaya dan pascapanen kopi*. Bogor: Pusat Penelitian dan Pengembangan Perkebunan. Bogor, 11-12.

Prastujati, A. U., Hilmi, M dan M. Habbib Khirzin. 2018. Konsentrasi starter terhadap kadar alkohol, pH, dan total asam tertitrasi (tat) whey kefir. *Jurnal Imu Peternakan Terapan*. 1(2):63-69. ISSN 2579-9479.

Prayitno, S. P., Guntoro. dan Utami, S. S. 2019. Jenis alat dan lama pengeringan terhadap kualitas mutu pada pembuatan teh cascara kopi. Seminar Nasional Hasil Pengabdian Masyarakat dan Penelitian Pranata Laboratorium Pendidikan Politeknik Negeri Jember.

Puspaningrum, D. H. D. dan Sumadewi, N. L. U. 2019. *Pengaruh metode pengeringan terhadap kandungan total fenol cascara kopi arabika (Coffea arabika L.)*. Skripsi. Bali: Universitas Dhyana Pura.

Putri, D. 2014. Aneka tanaman perkebunan. http://anekatanamanperkebunan.blogspot.com/2014/11/jenis-dan-karakteristik-kopirobusta.html. [Diakses pada tanggal 12 Maret 2021].

Putri, D. A. 2020. Cara membuat teh kombucha yang benar (dilengkapi kandungan nutrisi). https://doktersehat.com/cara-membuat-teh-kombucha/. [Diakses pada 14 Maret 2021 pukul 12:16].

Rahardjo, P. 2012. *Panduan budidaya dan pengolahan kopi arabika dan robusta*. Jakarta: Penebar Swadaya.

Risnandar, C. dan Fahmi, A. 2018. Kopi robusta. https://jurnalbumi.com/knol/kopi-robusta/. [Diakses pada 13 Maret 2021 pukul 16:32 WIB].

Rusdiana, F. F. 2017. Kombucha, minuman probiotik dari larutan teh. https://sith.itb.ac.id/. [Diakses pada 13 Maret 2021 pukul 18:51 WIB].

Sa'yidah, L. dan Lestari, K. A. P. 2020. Pengaruh Lama Pemanasan Terhadap Nilai ALT Bakteri Teh Kombucha. *Journal of Pharmacy and Science*, 5(1). P-ISSN: 2527-6328, E-ISSN : 2549-3558.

Sardjiman. 2011. *Belajar kimia organik metode iqro*. Yogyakarta: Pustaka Pelajar.

Sholichah, E., Apriani, R., Desnilasari, D., Karim A. M. dan Harvelly. 2019. Produk samping kulit kopi arabika dan robusta sebagai sumber polifenol untuk antioksidan dan antibakteri. *Jurnal Teknologi Pangan*.

Simanjuntak R. J. D., Mutiara, H. 2016. Pengaruh pemberian teh kombucha terhadappertumbuhan Salmonella typhi. *Jurnal Medikal Universitas Lampung*, 5, 48-54.

Slinkard, K. and V. L. Singleton. 1977. Total phenol analysis: Automation and comparison with manual methods. *American Journal of Enology and Viticulture*, 28, 49-55.

Soraya, N. 2008. *Isolasi kafein dari limbah teh hitam ctc jenis powder secara ekstraksi*. Skripsi. Bogor: Institut Tertanian Bogor.

Subeki., Winanti., Diki. dan Nauli, P. 2019. *Kandungan polifenol dan kualitas seduhan cascara (teh ceri kopi) fine robusta sebagai rintisan perusahaan pemula berbasis teknologi*. Seminar Nasional Tektan Polinela, Bandar Lampung 7 November 2019.

Subyekti, M. 2012. *Pengaruh suhu pengeringan dan proses blansing terhadap mutu tepung daun singkong (Manihot esculenta C.) dengan metode oven konveksi*. Skripsi. Universitas Padjadjaran.

Suhardini, P. N., dan Zubaidah, E. 2015. Studi aktivitas antioksidan kombucha dari berbagai jenis daun selama fermentasi. *Jurnal Pangan dan Agroindustri*, 4(1), 221-229.

Sulistyaningtyas, A. R. 2017. *Pentingnya pengolahan basah (wet processing) buah kopi robusta (Coffea robusta) untuk menurunkan risiko kecacatan biji*. Prosiding Seminar Nasional Universitas Muhammadiyah, Semarang 30 September 2017.

Sumihati, M., Widiyanto dan Isroli. 2011. Utilitas protein pada sapi perah friesian holstein yang mendapat ransum kulit kopi sebagai sumber serat yang diolah dengan teknologi amoniasi fermentasi (amofer). *Sintesis.* 15(1), 1-7.

Sun, T. Y., Li, J. S., Chen, C. 2015. Effects of blending wheatgrass juice on enhancing phenolic compounds and antioxidant activities of traditional kombucha beverage. *J Food Drug Anal*, 23, 709-718. doi: 10.1016/j.jfda.2015.01.009.

Urbahillah, A. 2018. *Karakteristik fisikokimia dan sensori kombucha cascara*. Skripsi. Universitas Jember.

Verawati, C. S. W. 2019. Perbandingan kadar fenol total dan aktivitas antioksidan ekstrak metanol daun kopi robusta (*Coffeea canephora*) dan arabika (*Coffeea arabica*). *Skripsi.* Yogyakarta: Universitas Sanata Dharma.

Villarreal-Soto, S. A., Beaufort, S., Bouajila, J., Souchard, J. dan Patricia Taillandier. 2018. Understanding Kombucha Tea Fermentation: A Review. *Journal of Food Science*, 83(3).

Wibawa, M. S. 2016. *Probiotik*. Skripsi. Bali: Fakultas Kedokteran, Universitas Udayana.

Widyotomo, S. 2012. Potensi dan teknologi diversifikasi limbah kopi menjadi produk bermutu dan bernilai tambah. *Review Penelitian Kopi dan Kakao*, 1(1): 63-80.

Wilson, C. 2018. *The clinical toxicology of caffeine: a review and case study*. Elsivier (Toxicology Reports), 5, 1140-1152.

Wistiana, D. dan Zubaidah, E. 2015. Karakteristik kimiawi dan mikrobiologis kombucha dari berbagai daun tinggi fenol selama fermentasi. *Jurnal Pangan dan Agroindustri*, 3(4), 1446-1457.

Yanti, N. A., Ambardini, S., Ardiansyah., Marlina, W. O. L. dan Cahyanti, K. D. 2020. Aktivitas antibakteri kombucha daun sirsak (*Annona muricata* L.) dengan konsentrasi gula berbeda. *Sainstek*, 8(2), 35-40.

Yuliandri, M.T. 2016. Cascara: teh dari ceri kopi. https://majalah.ottencoffee.co.id/cascara-teh-dari-ceri-kopi/. [Diakses pada 17 Maret 2021 pukul 06:34 WIB].

Zubaidah, E., Dewantari, F. J., Novitasari, F. R., Srianta, I., Blanc, P. J. 2018. Potential of snake fruit (salacca zalacca (gaerth.) Voss) for the development of beverage through fermentation with the kombucha consortium. *Biocatal Agric Biotechnol*, 13, 198-203. doi: 10.1016/j.bcab.2017.12.012.