Study on Fermentation of Raw Coffee Beans and Composting of Spent Coffee Grounds

Program of Biological System Sciences,
Graduate School of Comprehensive Scientific Research,
Prefectural University of Hiroshima

Doctoral Thesis

2021

Asmak Afriliana

Study on Fermentation of Raw Coffee Beans and Composting of Spent Coffee Grounds

A dissertation submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy (Ph.D) in

Biological Science

By

Asmak Afriliana

Graduate School of Comprehensive Scientific Research

Program in Biological System Science

Prefectural University of Hiroshima, Japan

2021

The dissertation submitted herewith is approved by the following members of the examination committee:

Prof. Hiroyuki Harada

Supervisor

Department of Environmental Science, Prefectural University of Hiroshima

---

Prof. Yoshiharu Mitoma

Supervisor

Department of Environmental Science, Prefectural University of Hiroshima

---

Assoc. Prof. Taizo Masuda

Supervisor

Department of Environmental Science, Prefectural University of Hiroshima

---

Prof. Tsutomu Morinaga

Thesis Examiner

Prefectural University of Hiroshima

Assoc. Prof. Yukihiro Yamamoto

Thesis Examiner

Department of Life Science, Prefectural University of Hiroshima

Contents

Composting of Spent Coffee Ground

Implementation of SCG compost
On Mustard Plant (Brassica Juncea L)

General Conclusion

List of Figures

List of Tables

ACKNOWLEDGMENTS

Alhamdulillahi rabbil ‘alamin, I expresses my highest gratitude to Allah subhanahu wa ta’ala for blessing, love, opportunity, health, and mercy to complete this thesis.

Behind the accomplishment of this thesis, there are support and assistance of many persons to whom I wish to express my heartily thankfulness. First and foremost, I would like to express my sincere gratitude with honor to my supervisor Prof. Hiroyuki Harada, Department of Environmental Science, Prefectural University of Hiroshima, Japan for nominating me as his student and for providing me the opportunity to work with a bunch of wonderful and talented researchers. I am indebted for his guidance, advice, constructive criticism to work and valuable suggestion to succeed against the arisen challenges during the whole period of conducting the research.

I also gratefully acknowledge Prof. Yoshiharu Mitoma, and Assoc. Prof. Taizo Masuda for discuss the tiniest details of any problem.

I thank the thesis committee members: Prof. Tsutomu Morinaga, and Assoc. Prof. Yukihiro Yamamoto. Their valuable feedback helped me to improve the dissertation in many ways. I also acknowledgment Islamic Development Bank (IDB) for awarding me the scholarship for carrying out this study.

I extend my sincere appreciation to all the lab-members with whom I have shared thoughts and views on work and of course joyous and poignant moments since April 2018.

I am thankful for the people who have influenced my educational and professional choices at home and abroad. I am forever indebted to my parents, Bisri Effendi, and Suwarni, who raised me with endless patience and provided me the best possible environment they could when it was most required. Lastly, I must acknowledge my loving, supportive and encouraging husband, Bayu Hidayat, who allowed me a protracted respite from raising our daughters, Fakhira and Hafshoh until the final stages of this work. The best husband who always prays and therefore everything becomes easy and this study can be completed on time.

Asmak Afriliana
Prefectural University of Hiroshima
February 2021

SUMMARY

Study on Fermentation of Raw Coffee Beans and Composting of Spent Coffee Grounds

By Asmak Afriliana

Supervisor: Professor Hiroyuki Harada

Department of Environmental Science, Prefectural University of Hiroshima

Refermentation technology is re-fermenting dry coffee beans that have not been fermented or the market term is inferior coffee bean using kefir starter in automatic fermenter. Re-fermentation of coffee bean can enhance the pleasant aroma until 23 compounds in 37°C for 18 hours. Some compounds group including of acid, alcohol, aldehyde and acetate groups were contributed to acidy, fruty, nutty and caramelly aroma. In this study, we continue our study to utilize SCG (Spent Coffee Ground) from refermented bean. Adding fungi starter such as *Penicillium sp* and *Aspergillus sp* with temperature control in composting SCG can improve quality compost produced, with the physical characteristics of compost black and crumb, and normal pH. While the chemical characteristics of compost produced is a C/N ratio below 10 with a far difference from the control. Compost is also richer in minerals, such as phosphorus, potassium, calcium, and magnesium, as well as rich in humic acid as shown from the results of the FTIR analysis. Germination Index of the compost sample with the addition of fungi activator (C2) is 191.86% greater than the commercial activator (C1) 183.88%. SCG compost which has been enriched with inoculant starter for composting can stimulate plant growth more when compared to commercial compost both organic and inorganic compost. Plants with the addition of SCG compost (C2) as much as 3% have the best results when compared to the addition of commercial compost (C1) and compost control (C0) in terms of plant physical and nutrients contained therein. Likewise, with the biomass produced. The results of the in vitro germination index analysis also proved that the fungi starter implemented in the Mustard plant had the best GI value, namely 200.4%.

CHAPTER 1

GENERAL INTRODUCTION

Chapter 1

General Introduction

1.1. THESIS RATIONALE

Coffee is an agricultural commodity that is in great demand by peoples. Coffee has become widespread throughout the world as a favorite beverage. This is because coffee has a special taste and often has an addictive effect for the drinker. In addition, there are many health benefits of coffee. That is the main reason researchers are competing to do research on coffee. In producing countries, fruit cultivated in coffee trees harvested, subjected to dry refining or wet refining, and then exported as raw beans. Dry refining involves sun drying the harvested fruits and removing the raw beans from a completely dry state. In wet refining, the peel and pulp are mechanically peeled off and immersed in water to decompose the sticky substances that adhere to the raw beans due to the action of microorganism. In consuming countries, raw beans are roasted to make roasted beans. To produce good quality coffee flavor, researchers work hard to carry out processing engineering, such as optimization of the coffee processing method where the main key to taste is the fermentation process. The following are the stages of the coffee refining process, in Figure 1.1.

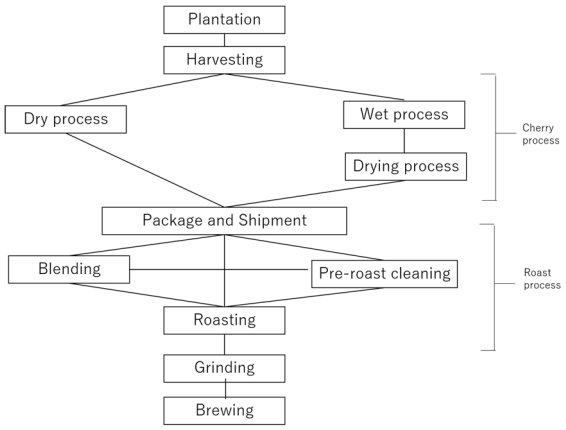


Figure 1.1. General Scheme of Coffee Processing

Therefore, in this doctoral thesis study, fermentation processing engineering was also studied, which is a relatively new technique, namely the coffee bean refermentation technique to improve the taste quality of coffee. In addition, we also studied the utilization of Spent Coffee Ground (SCG). The term referenced which means re-fermenting dry coffee beans that have not been fermented or the market term is inferior coffee bean. The purpose of this refermentation technique is to improve the taste quality of the inferior coffee beans that are already on the market. From dry coffee beans

that have low flavor quality, we can still improve their quality with this technique. Usually in the market, the inferior coffee is sold so low price that it is very detrimental to farmers.

The fermentation process usually requires a medium as nutrients for the growth of microorganisms. In coffee, coffee fermentation techniques are usually carried out on coffee cherries, which are fruit that are still intact or coffee beans that have been peeled or dipped and still contain mucilage as a medium or nutrition for microorganisms to carry out the fermentation process. Mucilage enveloping the coffee beans, Figure 1.2 shows the anatomy of the coffee cherries. Whereas in the refermentation technique, because the fermentation process is carried out on dry coffee beans, it is necessary to recondition the beans so that they are ready for fermentation. The coffee beans are soaked in water until the water content is around 60%, then a starter is added and fermented at temperature of about 21, 37, and 47^0C in a controlled reactor. In this study we use kefir or fermented milk beverage containing lactic acid bacteria and yeast as starter cultures. In addition, in this study, lactose was added to increase nutrition for microorganisms and improve flavor. The dried beans were roasted and crushed to extract coffee, and sensory test and chemical analysis were performed. The pyrazine and aldehyde components increased to more than 20% each. These were factors that made smell of chocolate and nuts by expert panelist. In addition, this study will add references to coffee fermentation techniques. The results of the analysis using this technique were able to increase the score by about 4 due to the change in these chemical composition.

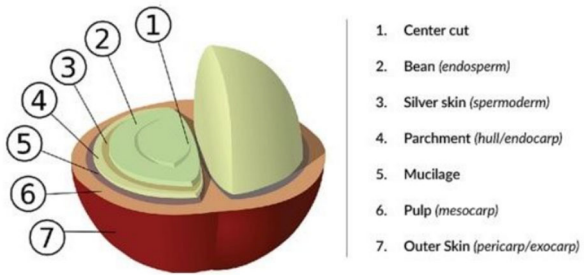


Figure 1.2. Anatomy of Coffee Cherry (Schuet, 2012)

One of the positive effects of fermentation apart from taste is nutritional. Coffee fermentation techniques are proven, can reduce caffeine levels in coffee beans. Likewise with coffee refermentation techniques. In Robusta coffee, caffeine drops from around 2-3% to around 1%. As we know, excessive amounts of caffeine in the body will actually have an effect that is not good for health. Although indeed some antioxidant compounds also go down, such as polyphenol compounds which actually have health effects for the body. However, because in the refermentation technology with kefir starters and combined with the use of a reactor with temperature control so that the fermentation process is faster, around 18 hours with produce 23 volatile compound, the decrease in

these antioxidant compounds can be minimized. In this study we also analyzed the total polyphenol content in fermented coffee beans.

Coffee grounds from the results of this refermentation technique, with the minimum content of caffeine and polyphenols, have potential if implemented for plant growth. Apart from of course the potential content in it, such as almost 99%, is organic matter, carbon, 2% nitrogen, 0.2% phosphoric acid and 0.3% potassium which is very good for plants. This is also the background of our study in chapter 3, namely regarding the use of coffee grounds as organic compost as well as the addition of a starter. Previous research, using coffee grounds directly to plants requires a long time for plant growth. This is because coffee grounds contain germination inhibitors such as caffeine and polyphenols. The composting process using starter fungi (*Aspergillus* and *Penicillium*) and combination with the optimum temperature of 30^0 Celsius can reduce carbon and increase nitrogen, decompose macromolecule content such as lignocellulose and protein. The decrease in lignin is said to be correlated with the increase in humic acid in the compost. In addition, the composting process can take place faster. In this study, the composting process for a month can produce compost that is in accordance with the standards. Finally, in this chapter we also analyze the germination index of radish plants to see the toxicity of compost. The resulting values are in the range of 180-190%. Previous research said that if compost has a germination index above 80%, it means that the compost is ripe and not toxic to plants.

In the next chapter, chapter 4, we studied the effect of SCG compost on mustard plant seedlings. The analysis of the total number of microbes showed that the number of microorganisms, especially fungi in the compost, was still very large, around 6.94 log cfu / ml (compost with the addition of starter fungi / C2). These fungi can function as Plant growth Promoting Fungi (PGPF) which has been proven in vitro on mustard plants to stimulate plant root growth and germination in plant seeds. To further deepen the effect of SCG compost on plants, we did pot treatment then carry out physical and chemical or nutritional analysis in the plants. Such as stem length and leaf width, as well as macro mineral content such as phosphorus, potassium, nitrogen, magnesium, and calcium. We compare our compost samples with commercial compost, both in the form of solids (organic compost) and commercial compost in the form of liquid (inorganic compost).

SCG compost which has been enriched with inoculant starter for composting can stimulate plant growth more when compared to commercial compost both organic and inorganic compost. This is evidenced by the longer the plant stem and the wider the leaves. In addition, the results of plant macronutrient content analysis also showed that the addition of compost during seeding can improve plant nutrients such as phosphorus, nitrogen, potassium, and other macro minerals so that it had a good impact on plant growth.

1.2. AIMS AND OBJECTIVE

The objectives of this study was to studies of fermentation of coffee bean and composting of spent coffee ground. Related to fermentation of coffee bean, we have been studied the optimal temperature and time for fermentation of inferior coffee beans uses kefir starter based on the results of the cup test or sensory test. In addition, to determine the changes in volatile compounds of after fermentation. The final objective of this research is to improve the quality of the inferior coffee bean by using a refermentation with kefir as starter cultures. To get optimal results, we experiment the fermentation process which carried out in a reactor with a controlled temperature of 27, 37, 47^0 Celsius for 0, 6, 12, 18 hours anaerobically. Stirring is done automatically in the reactor. The starter used was 1%, besides that, 2% lactose was also added. Some of the parameters analyzed were pH, Lactic Acid Bacteria (LAB) population, changes in chemical content (glucose, caffeine, protein), and most importantly sensory analysis by expert panelists and changes in volatile compound by Gas Chromatography-Mass Spectrometry (GC-MS).

The coffee grounds from the fermented coffee beans are then composted with the addition of a specific starter and temperature control. The starters used are *Aspergillus* and *Penicillium sp* at a temperature of 30^0 Celsius and composting is carried out aerobically in a container equipped with a hole for circulation water. Stirring is done manually every day. Some of the parameters analyzed are pH, electric conductivity (EC), Carbon/Nitrogen (C / N) ratio, % of decompose macromolecule such as fiber and also protein. We also analyzed the content of several macro minerals such as potassium, calcium, manganese, and phosphorus. Then finally we also studied the compost germination index applied to radish plants and the content of fungal groups using Fourier Transform Infra Red (FTIR). The purpose of composting in this way is to speed up composting time, and improve the quality of the resulting compost, so that it is suitable for application in horticultural plants, namely normal pH, low C / N ratio, high nitrogen and other important minerals which are very useful for plant growth. SCGs compost that rich of fungi apply for horticultural plants, namely Japanese mustard plants. Then the plants were also physically analyzed which included biomass, leaf width and stem height. As well as other analyzed parameters are the nutrient content in it. The purpose of this final stage of research was to determine the effect of SCGs compost on the growth of mustard plants.

1.3. SUMMARY AND OUTLINE OF THE THESIS

This manuscript consists of 5 chapters. In chapter 1, it summarized the background of research and explained the design of the dissertation structure. In chapter 2, it was explained about coffee refermentation technology with the addition kefir as starter cultures and temperature control in the reactor to improve the taste quality of coffee beans, as evidenced by the results of taste tests by expert panelists and changes in aroma or volatile compound analysis by GC-MS. In chapter 3, it was explained about composting coffee grounds with the addition of a starter function and temperature control to improve the nutritional quality of the compost produced, as evidenced by several chemical parameters and analysis of the germination index on a horticultural plant. In Chapter 4, a study on the implementation of SCGs compost that rich on fungi to Japanese Mustard plant and it is effect to physic and nutrients of plants.

Each chapter consist of introduction, literary review, experimental methods, results and discussion, and conclusion. The overall conclusion of the work and future directives for further works are presented in Chapter 5.

A list of publications resulting from these works is given in Appendix A. in Appendix B a list of contributions to scientific forum is placed whereas Appendix C provides curriculum vitae of the author.

CHAPTER 2

Refermentation Technology on Robusta Coffee Bean (*Cafea Canephora*)

Chapter 2

2.1.Background Research

In this chapter, we study about coffee processing, especially in the fermentation process. This is because fermentation is very influential on the taste of coffee. The main purpose of fermentation is to remove the mucilage layer attached to the hard skin of the coffee bean. The mucilage layer consists of 84.2% water, 4.1% sugar, 8.9% protein, 0.91% pectic acid, and 0.7% ash (Yusianto and Nugroho, 2014). Mainly simple sugar and pectin compounds which are converted into alcohol and organic acids by microorganisms during fermentation so that they can lower the pH of the beans and change the texture of the mucus layer to be easy to wash and remove (Correa et al., 2014).

In addition, fermentation also affects the flavor formation of coffee beans, especially to reduce the bitter taste and encourage the formation of a mild impression on the flavor of the brew. The main factors determining the quality and fermentation taste of coffee beans are the kind of coffee, temperature and pH of fermentation, fermentation time, and use of fermentation containers, especially temperature and fermentation time (Masoud et al., 2006; Yusianto and Widyotomo, 2013). In previous studies, kind of coffee that is often analyzed regarding the fermentation process is Canephora coffee. This is because Canephora coffee has a lower coffee quality compared to Arabica and Excelsa or Liberica coffee types. Besides that, Canephora coffee also has higher caffeine levels, which is 2-4% (Gaibor et al., 2020).

Previous research related to other coffee fermentation is temperature, fermentation time, and type of fermentation container (Sinaga, 2018; Neto et al., 2018). Research on the types of starter used during fermentation has also been widely studied (Nava et al., 2011; Usman et al., 2015; Tika et al., 2017). Previously, people were familiar with coffee fermentation as a natural or dry process, wet process, semi wet process, honey process and carried out aerobically. Recently, coffee fermentation techniques have also begun to develop, for example carbonic maceration which was introduced in 2015 by Sasa Setic as the winner of the world barista championship (Gudi, 2017), as well as anaerobic fermentation (Davids, 2019). The novelty of the fermentation technique is when the fermentation process takes place the coffee cherries are not depulped. Whole coffee cherries are put into the fermentation tank and pumped to hold the carbon dioxide. The pump is then put into a container filled with water (Gudi, 2017). Meanwhile, anaerobic fermentation is the same, whole coffee cherries are put into the fermentation tank and it is ensured that there is no oxygen in the tank (Davids, 2019). This fermentation technique produces a more unique coffee flavor.

However, never been studied regarding of using kefir as a starter and combination in automatic fermentor. Neto et al., 2018 studied the use of fermentation reactors with the addition of lactobacillus plantarum to produce high quality seeds. In this study, a starter from kefir was used. Kefir seed contain a mixture of bacteria (*Lactobacillus, Lactococcus* and *Acetobacter* species) and yeast. According to (Leite et al., 2013), the identified types of microbes in kefir are *Leuconostoc mesenteroides* (29%), *Lactococcus lactis* spp. (5%), *Lactococcus lactis* ssp. *cremoris* (45%), *Acetobacter lovaniensis* (10%), and *Saccharomyces cerevisiae* (11%). The fermentation process is carried out in a reactor with temperature control. In addition, lactose is also added to add nutrients to the growth of microorganisms in the reactor. During the coffee bean fermentation, these microorganisms inside kefir, which are lactic acid bacteria and yeast, will remodel the mucus layer into organic acids (Yusianto and widyotomo, 2013). These organic acids play a role in the sour taste of coffee brewing. The use of a starter from several microorganisms is expected to maximize fermentation, so that fermentation can take place faster with good quality.

This fermentation technique is called Refermentation Technology. This technique also tends to be new, because in this study the fermentation technique was carried out on dry coffee beans, or coffee beans that were not fermented before. The background of this technique is that in Indonesia, the fourth largest coffee producer in the world with the most types of coffee is Canephora coffee (Rahmanullah, 2017). On the market, we can still find a lot of inferior coffee beans. The quality of that coffee beans is very low. So that the selling price of coffee is also very low. Therefore, it is necessary to re-ferment the coffee beans to increase the taste and sale value. This study aims to study the effect of applying refermentation technology of dry coffee bean using kefir as starter in a controlled reactor on the quality of the coffee beans produced.

2.2.Literary Review

2.2.1. Coffee

The genus Coffea belongs to the family *Rubiaceae*. This family comprises many genera including *Gardenia, lxora, Cinchona (quinine)* and *Rubia*. The two main species of coffee tree cultivated on a worldwide scale are *Coffea arabica* and *C. canephora* var. *robusta*. Minor cultivated species include *C. liberica* and *C. excelsa*, which are mainly restricted to West Africa and Asia, and account for only 1–2% of global production (Wintgens et al., 2008). The coffee plant takes approximately 3 years to develop from seed germination to first flowering and fruit production, Figure 2.1.

Figure 2.1. (a) Coffee Seedling, (b) Adult Coffee Plant, (c) Ripe Coffee Cherries, (d) Coffee Bean (1) Arabica, (2) Liberica, (3) Canephora or Robusta (Rodrigues et al., 2013).

There are 7 layers to the bean cherry, such as centre cut, bean, silver skin, parchment, mucilage, pulp, and outer skin, Figure 2.2. There are two coffee beans inside the coffee cherry. The beans are separated by pulp. The description of each layer, below (Hoffmann, 2014).

1. Center Cut: Is what some would probably describe as the heart of the coffee bean. This is the part where all drinkers are looking for.
2. Endosperm: This is what gives us that delectable coffee flavor and beautiful aroma scent in the roasting process
3. Silver Skin: This skin remains wrapped around the bean after the peeling process is complete.
4. Parchment: Actually looks like parchment paper when dried. This is the beans protector
5. Mucilage: This is the layer between the pulp and parchment. This layer just like the pulp is rich with sugars, and pectin.
6. Pulp: Also known as Mesocarp. This substance is rich in sugars, and pectins.
7. Outer Skin: Is the outside skin known as the (cascara) of the cherry. It is when the color of the cherry turns red when it is at its prime and ready for picking.

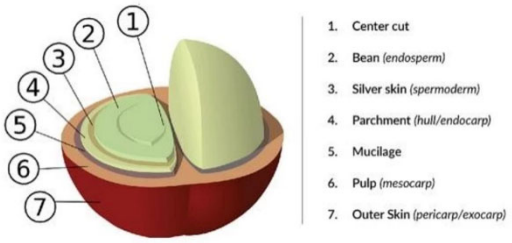


Figure 2.2. Anatomy of Coffee Cherry (Schuett, 2012).

The flesh of the fruit that has been ripe is slimy and contains sugar compounds so it tastes sweet (Tables 2.1 and 2.2). The mucilage layer is firmly attached to the surface of the horn skin. The mucilage content in young fruit is very little, while in overripe fruit, reduced because it has broken down naturally. Therefore, when harvested coffee cherries must be in the right ripe condition. So that the mucilage content in it is a lot. This mucilage is nutrition for the development of microorganisms during the fermentation process.

Table 2.1. Chemical Composition of Coffee Cherries (Afriliana, 2018)

Component	(%)
1. Water	42.66
2. Fiber	27.44
3. Glucose	9.46
4. Tannin	8.56
5. Mineral	3.77
6. Lipid	1.18
7. Volatile compound	0.11

Table 2.2 Chemical Composition of Mucilage (Afriliana, 2018)

Component	(%)
1. Water	84.2
2. Protein	8.9
3. Glucose	4.1
4. Peptic acid	0.9
5. Ash	0.7

The demand for coffee is always increasing from year to year. Coffee is the third most traded commodity in the world, with about 500 million cups per year. World coffee consumption in 2019

reached 168.49 million bags, increase 0.33% from previous year (ICO, 2020). Therefore, research on coffee continues to be conducted to produce superior quality beans, namely whole coffee beans with high flavor.

Green coffee beans are contain soluble carbohydrates, such as the monosaccharaides fructose, glucose, galactose and arabinose, the oligosaccharides sucrose, raffinose and stachyose, and polymers of galactose, mannose, arabinose and glucose. Soluble carbohydrates act binding aroma, stabilizing foam, sedimenting and increasing viscosity of the extract. In addition, non-volatile aliphatic acids (citric, malic and quinic acids) and volatile acids are also present (such as acetic, propanoic, butanoic, isovaleric, hexanoic acids). Oils and waxes are also important constituents, accounting for 8 to 18% of the dry mass, together with proteins and free amino acids (9-12% w/w) and minerals (3-5% w/w). (Arya & Rao, 2007; Belitz et al., 2009; Clifford, 1985a; Gonzalez-Rios et al., 2007). Table 2.3 shows the chemical composition of green Arabica and Robusta beans.

There are many compounds in coffee that are often thought to have implications upon human health. These include caffeine, micronutrients and chlorogenic acid. The coffee beverage is rich in biologically active substances such as nicotinic acid, trigonelline, quinolinic acid, tanninc acid, pyrogolic acid and caffeine (Minamisawa et al., 2004). Sometimes coffee quality became low due to the hydrolysis of triacylglycerols (the major constituent of coffee lipid) releasing free fatty acids which are oxidized at the time of storage. Multon et al (1973) reported that free amino acids and sugars are degraded while lipids are oxidized and produce off flavour at the end of the storage after drying.

Table 2.3. Chemical composition of green Arabica and Robusta coffee beans (Ghosh, 2014)

Component	Arabica Coffee (g/100g)	Robusta Coffee (g/100g)
Polysaccharides	49.8	54.4
Sucrose	8.0	4.0
Reducing sugars	0.1	0.4
other sugars	1.0	2.0
Lipids	16.2	10.0
Proteins	9.8	9.5
Amino acids	0.5	0.8
Aliphatic acids	1.1	1.2
Quinic acids	0.4	0.4

Component	Arabica Coffee (g/100g)	Robusta Coffee (g/100g)
Chlorogenic acids	6.5	10.0
Caffeine	1.2	2.2
Trigonelline	1.0	0.7
Minerals (as oxide ash)	4.2	4.4
Volatile aroma	traces	traces
Water	8 to 12	8 to 12

2.2.2. Coffee Processing

One of the reasons coffee is expensive is due to the large processing cycle that coffee goes through. Coffee processing cycle is summarized in Figure 2.3.

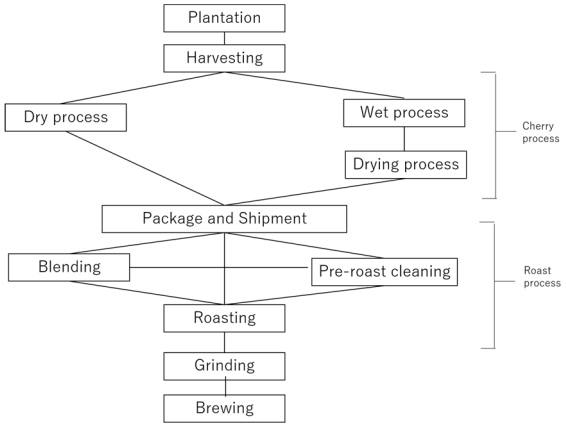


Figure 2.3. Coffee Processing

Green coffee is produced either by dry processing or by wet processing. After harvesting, the coffee fruits are separated from the pulp, which is carried out by dry or wet processing (Clarke & Macrae, 1987; Illy & Viani, 1995). The dry process is simple and inexpensive. The whole cherries are dried under the sun in open air, followed by the separation of the hull (dried pulp and parchment) for getting the green beans. On the contrary, the wet process requires more care and investment, but results in a superior coffee quality. In the wet process, the pulp of the coffee cherries, which is made up of exocarp and mesocarp, is removed mechanically, but the parchment remains attached to the beans. After drying either under the sun or in a dryer, the parchment is removed to produce the green coffee beans.

2.2.3. Coffee Fermentation

The fermentation process is one stages in wet processing of coffee beans. This process not only for degradation of its mucilage layer remaining on the surface of the horn skin, but also occurs very useful in chemical events formation of flavor characters, namely formation flavor precursor compounds, such as organic acids, acids amino, and reducing sugars (Avallone, Brillouet, Guyot, Olguin & Guiraud, 2002; Jackels & Jackels, 2005; Redgwell & Fischer, 2006; Lin, 2010). Therefore, the fermentation process is an important stage in wet coffee processing, remember positive influence for the improvement of flavor (FAO, 2004; Mondello et al., 2005; Singh & Singh, 2013; Correa et al., 2014).

Wet fermentation is carried out using water. This process is done in special tanks has been equipped with drains. The coffee beans that have been processed by a pulping machine are then flowed into the tub through a water channel. This way, some of the mucus layer covering the surface of the seeds will disappear. The length of the fermentation process is influenced by the type of coffee, the temperature and humidity of the environment and the thickness of the coffee bean pile. The fermentation reaction starts at the top of the pile because there is enough oxygen. The mucilage layer will peel off and the reaction compounds move down to the bottom of the tub and accumulate at the bottom of the tub. In order for fermentation to take place evenly, the coffee beans in the tub need to be turned at least once a day. The end of fermentation is marked by the peeling of the mucus layer that covers the horn skin. Fermentation can be done wet and dry process (ICCRI, 2008). Wet processing by fermentation is intended to form the distinctive flavor elements of coffee.

Dry fermentation is done by means of pile the coffee that has just come out of the peeler in the shade for 2-3 days. The coffee pile is closed to keep it moist so the fermentation process goes well. In general, with the longer fermentation, the acidity of the coffee will increase. This is caused by the formation of aliphatic acids during the fermentation process. If the fermentation time is extended it will be continues to change the chemical composition of coffee beans, where Aliphatic acids will change into carboxylic acid esters which can cause fermentation defects with a bad taste (Sulistyowati and Sumartona, 2002).

The bacteria found in wet fermentation are aerobic bacteria such as *Klebsiella ozaenae*, *K.Oxytoca, Erwinia herbicola, E. disolvents, Hafnia spp., Enterobacter aerogenes* and lactic acid bacteria such as *Leuconostoc mesenteroides, Lactobacillus brevis*. Yeasts that play a role during fermentation are *Kloeckera apis apicualata, Candida guilliermondii, C. tropicalis, C. parapsilosis, Cryptococcus albidus, C. laurentii, Pichia kluyveri, P. Anomala, Hanseniaspora uvarum, Saccharomyces cerevisiae, Debaryomyces hansenii, Torulaspora delbrueckii* and *Rhodotorula*

mucilaginosa. The microflora is microflora which will degrade mucilage. During fermentation the pH decreases and the yeast group was the dominant microflora that was resistant to levels acidity (Lee et al., 2015).

2.2.3.1. Chemical Changes during Fermentation

Changes that occur during fermentation according to Schwan and Fleet (2015):

1. Breaking of the mucilage component

Fermentation will result in the breakdown of protopectin components by enzymes catalase from coffee cherries. This solution will run fairly quickly at a pH of 5.5-6.0 and when the pH is lowered to 3.65, the breakdown will be 3 times faster. According to Mulato et al. (2006), in fermentation can be added 0.025 percent. The pectinase enzyme produced from the isolation of a type of bean with additions and the temperature is raised slightly, fermentation can last for 5 up to 10 hours. Natural fermentation takes 10 about up to 36 hour. Meanwhile, natural fermentation takes about 36 hour.

2. Breakdown of Sugar

The sugar content in the pulp will increase rapidly during the process fruit ripening. During fermentation, sugar is used by microorganisms as a growth substrate. Sugar as a substrate for microorganisms will change into alcohol by yeast, Figure 2.4.

$$C_{12}H_{22}O_{11} + H_2O \longrightarrow 2C_6H_{12}O_6 + 4.5\ kcal$$

$$C_6H_{12}O_6 \xrightarrow{yeast} 2C_2H_5OH + 22.3\ kcal$$

Figure 2.4. Breakdown of sugar into alcohol (Soenaryo, 1978)

In the next fermentation, the alcohol formed will be broken down into acetic acid by the activity of acetic acid bacteria. Image of solving can be seen in Figure 5.

$$C_2H_5OH+O_2 \xrightarrow{Acid\ bacteria} CH_3COOH+H_2O+ 188.6\ Kcal$$

$$CH_3COOH+2O_2 \longrightarrow 2CO_2+2H_2O+ 209.4\ Kcal$$

Figure 2.5. The reaction of breaking alcohol into acetic acid by acetic acid bacteria (Fauzi, 2008)

3. Browning

Color change (browning) during fermentation is due to presence polyphenol oxidation. This browning is undesirable because the coffee beans will brown and less attractive, but browning can be prevented by use of washing water which is alkaline. According to Yusianto and Widyotomo (2013), using too high a temperature can cause defective seed content brown in color compared to

coffee fermented at room temperature. Brown beans are a category 1 physical defect which has a direct effect to the flavor of brewing coffee.

4. Decrease Caffeine level

The longer the fermentation time, the caffeine concentration in coffee getting smaller. This is due to the degradation of caffeine by microorganisms become uric acid and biomass during fermentation (Farida et al., 2013). Based on research conducted by Hanifah and Kurniawati (2013) which ferments the coffee using lye and yeast solution obtained results that fermented coffee has decreased caffeine content by 0.6%.

5. Decrease in pH

Fermentation can cause the pH of the coffee beans to decrease as it occurs breakdown of sugar or caffeine which will be converted into uric acid or other acidic compounds such as lactic, acetic, butyric and propionic acids. Based on the pH provisions of the Quick Reference Food Charts, food pH limits and the drinks are 4-9. If the coffee acid level is too high it can be done reduction of acidity (de-acidification), namely by washing (Farida et al., 2013).

2.2.4. Kefir

According to Otes and Cagindi (2003), kefir is a popular traditional drink in the Middle East. This drink is produced from a fermentation process using kefir seeds. Kefir seeds look like corals or small clumps of cauliflower which Kefir can be made from various types of milk, namely cow, goat, sheep, coconut milk, rice juice and soybean juice.

Kefir making both traditionally and industrially. Traditional processing is the addition of kefir seeds directly. Raw milk is brought to a boil and then cooled to a temperature of 20-25ºC. Cooked milk is added with inoculant as much as 2-10% (generally 5%) and incubated for 18-24 hours at 20-27ºC. Fermentation is stopped by separating the kefir seeds from the milk by filtering them using a sieve. The separated kefir seeds can be used in the next inoculation (Otes and Cagindi, 2003).

Kefir is a probiotic beverage, so the expected fermentation result is the number of microbes contained in kefir. Probiotic have health effects. Based on the FAO / WHO (in Leite et al., 2013), the recommended minimum number of microbes in kefir is total bacteria 10^7 cfu / g and yeast 10^4 cfu / g.

The results of research conducted by Leite et al. (2013) regarding changes in carbohydrates during kefir milk fermentation, namely the significant decrease in lactose after fermentation for 12 hours (43.4 to 32.4 mg / ml). The decrease in lactose is offset by an increase in the amount of glucose and galactose. In addition, the pH value decreased from 6.55 to 4.31 due to the production of organic acids during fermentation. Lactic acid increased after 24 hours of fermentation with the amount

from 7.38 mg / ml increasing to 9.54 mg / ml. Acetic acid was also produced in kefir fermentation after 12 hours of fermentation with the amount increasing from 0.93 to 1.16 mg / ml. Ethanol compounds were also produced in kefir fermentation after 18 hours of incubation. The increase in ethanol compounds from 0.14 mg / ml to 0.32 mg / ml.

2.2.5. Lactic Acid Bacteria for Coffee Fermentation

Lactic acid bacteria (LAB) are a microbial group of substantial economic importance, used extensively as a starter culture in the production of fermented foods. Metabolic-based definition classifies LAB by the formation of more than 50% lactic acid as end product of carbohydrate utilization, besides being gram-positive, non-motile, and non-spore-forming bacteria with cocco-bacilli or rods morphology (Holzapfel, 2014).

In addition to mucilage removal, LAB have been associated with the generation of bioactive compounds related to coffee quality. The flavor-forming capability of 'wild-type' LAB cultures in coffee fermentation has gained increased interest due to the diverse aromas such strains are capable of impacting. Some LAB-derived metabolites, such as esters, ketones, higher alcohols, and aldehydes, can influence sensory attributes of coffee beverages with distinct floral, fruity, and buttery perceptions (Wang et al., 2019 and Pereira et al., 2016).

The phylogenetic relationship of coffee-related LAB species isshown in Figure 1. They are divided into four clades (*Leuconostoaceae, Lactobacillaceae, Streptococcaceae, and Enterococcaceae* families) occurring in the taxonomic genera Leuconostoc, Fructobacillus, Weissela, Lactobacillus, Pediococcus, Lactococcus, and Enterococcus. It is plausible that LAB initially colonized coffee fermentation from surface of coffee fruits. However, soil, water used in the processing, coffee equipment, and insets are also potential sources of LAB (Carvalho et al., 2018; Chen et al., 2007; Endo et al., 2013). After assessing the fermentation process, LAB quickly proliferate and reached population of 10^8cells/mL within 20 hours (Avallone et al., 2002).This accentuated growth is related for LAB adaptability to the environment and stress factors of coffee processing, such as pH variation, sugars availability, and competition with other microorganism (Mayo et al., 2010).

2.2.5.1 Metabolism of LAB in coffee fermentation

The abundant sugar content present in coffee pulp, including pentoses (xylose, ribose, and arabinose), hexoses (glucose, fructose, galactose, and mannose) and polysaccharides (pectin and cellulose), are primary carbon and energy sources for LAB growth (Figure 2.6). Homofermentative LAB species, such as *Lcc. lactis, P. pentosacesus, E. faecalis,* and *Lcb. hordei*, ferment sugars by the Embden–Meyerhoff–Parnas (EMP) pathway to pyruvate, which is converted into lactic acid by

lactate dehydrogenase (Endo et al., 2014; Zhang et al., 2019; De Bruyn et al., 2017; Leong et al., 2014). However, it is possible to suppose that, under coffee-related stress conditions (e.g. carbon limitation and acid environment), these homofermentative species can shift into a mixed-acid metabolism (Mayo et al., 2010).

Heterofermentative LAB, such as *Leu. mesenteroides, Leu. citreum,* and *Lcb. brevis*, are also commonly found in coffee fermentations (De Bruyn, 2017; Leong et al., 2014; Avallone et al., 2002). These LAB species are able to catabolize pentoses and hexoses present in coffee pulp into a vast range of end-metabolites, including lactate, acetate, CO_2, and ethanol, via phosphoketolase or pentose phosphate pathways pathway. Lastly, LAB are able to promote the breakdown of complex carbohydrates through the production of different hydrolytic enzymes (e.g. pectin lyase, pectin methylesterase, and exo-polygalacturonase) and acidification process (Avallone et al., 2002; Silva et al., 2013). The hydrolysis of pectin releases simple sugars (glucose, rhamnose, xylose, galactose, arabinose, and Dgalacturonate) as an additional carbon source for LAB growth (Pereiraet al., 2016; Avallone et al., 2002). In addition to sugars, LAB species have the capability of metabolizing citrate present in high concentration in the coffee pulp. The metabolism of citrate by LAB occurs through three processes: (i) citrate transport, (ii) conversion of citrate into oxaloacetate, and (iii) conversion of oxaloacetate into pyruvate and CO_2 (Garcia et al., 2016). This process leads to the production of 4-carbon, flavor-active compounds, including diacetyl, acetoin, and 2,3-butanediol. In addition, accumulation of pyruvate in an acidic environment such as coffee pulp favors the production of an acetolactate, a precursor of 4-carbon compounds [34]. *Leuconostoc* and *Lactococcus* species found in coffee fermentation are reported as presenting accentuated citrate metabolism (Drider et al., 2004; Sanchez et al., 2009).

Amino acid catabolism has an important role in LAB physiology for obtaining energy in nutrient-limited conditions and participating in pH homeostasis (Mayo et al., 2010).In addition, LAB are auxotrophic for a variable number of amino acids, depending on a rich environment for their growth. Coffee pulp provides such conditions due a rich constitution in leucine, valine, phenylalanine, threonine, and isoleucine (Pereiea et al., 2019). The catabolism of amino acids has implications with the formation of low-molecular weight compounds, such as and aldehydes, esters, carboxylic acids, and higher alcohols (Figure 2). For instance, strains of *Lcb. plantarum* are able to produce phenylacetaldehyde, phenylacetate, and phenylethanol during the phenylalanine catabolism, while *Lcc. lactis* strains are capable to metabolize leucine into 3-methylbutanal, 3-methylbutanol, and 3-methylbutyric acid (Vermeulen, 2006; Smit et al., 2005).

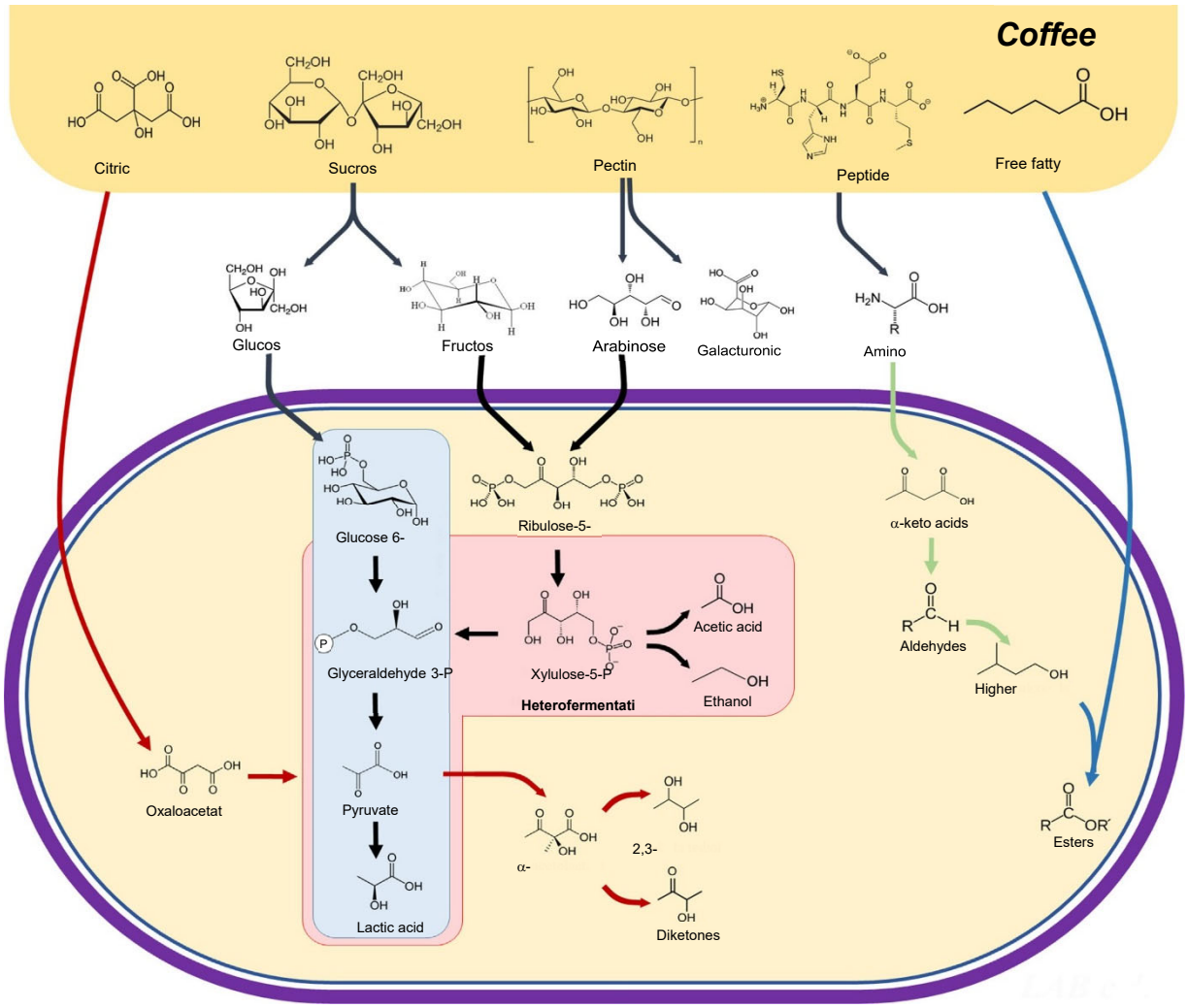


Figure 2.6. Schematic representation of the major metabolic pathways and metabolites generated by LAB during natural coffee fermentation from existing precursors (Gilbert, 2020).

2.2.5.2. Impact on Beverage Quality

The complex metabolism of LAB yields flavour active metabolites that impact on coffee quality (Table 2.4). The intense diffusion of lactic acid during fermentation process promotes the modification in sensorial perception of acidity and body of coffee beverages (Carvalho, 2018; Pereira, 2016). From citrate metabolism, the production of 2,3-butanedione and acetoin confers buttery-like aroma in coffee beverages (Wang et al., 2019; Pereira et al., 2019). In addition, a vast range of LAB-derived metabolites associated with the catabolism of amino acids, specifically ethyl propionate, ethyl acetate, acetaldehyde, phenyl ethanol, and pheny-lacetaldehyde, are known for increasing fruity and floral perceptions in final beverage (Pereira et al., 2019).Undoubtedly, aroma is one of most important characteristics that contribute to the quality of coffee. As in many foods, coffee aroma is composed by over 1000 different volatile organic compounds with concentrations that can

vary between 845 and 1239 ppm (Lee et al., 2015). The balance and interaction of all of them determine the coffee aromatic quality. The use of LAB cultures was demonstrated to be favourable for the production of coffee with distinctive sensory profiles. Coffee beverages with high acidity and fruity and floral perceptions were produced by the use of the *Lcb. plantarum LPBR01* (Pereira et al., 2016), while caramelic and burnt characteristics were the sensory attributes for *Lcb.rhamnosus HN001* inoculated treatment. These coffee beverages can strategically be used to supply specific coffee consumer regions or be used in blends to achieve desirable acidity and distinctive flavour.

Table 2.4. Main metabolites produced by coffee-related LAB and their metabolic pathway and sensory influences (Gilberto et al., 2020).

Metabolite	Sensorial description	Metabolic pathway	Producing LAB
Lactic acid	Sour, acid	Embden–Meyerhoff–Parnas, phosphoketolase or pentose phosphate pathways	All species
Benzaldehyde	Fruity, cherry	Tryptophan and phenylalanine catabolism	*O. oeni, Lcb. plantarum*
Acetaldehyde	Fruity	Threonine catabolism	*Lcb. brevis, Lcb. casei delbrueckii subsp. bulgaricus and S. thermophilus*
3-Methyl-butana	Chocolate, nutty, leafy, cocoa	Branched-chain amino acids catabolism	*Lcc. lactis, Lcb. helveticus*
2-Nonenal	Fatty with a citrus nuance	Lipid oxidation	*Lcb. sanfranciscensis and Lcb. reuteri*
Nonanal	Citrus, with a fresh green lemon peel-like nuance	b-Oxidation of unsaturated free fatty acids	*Lcb. sanfrancisco, Lcb. rhamnosus,*
Decanal	Orange peel, citrus, and floral	b-Oxidation of unsaturated free fatty acids	*Lcc. lactis ssp. cremosis, Lcc. lactis ssp. lactis*
Ethyl acetate	Fruity, grape	Esterefication reaction between alcohols and free fatty acids	*Lcc. lactis ssp. lactis, Lcb. rhamnosus, Lcb. paracasei ssp. paracasei, Lcb. plantarum, Leu. lactis, Pediococcus sp*
Ethyl butanoate	-	Esterefication reaction between alcohols and free fatty acids	*L. plantarum, L. rhamnosus, L. fermentum, L. helveticus, L. casei Pediococcus sp., Lcc. lactis, Lcc. lactis ssp. lactis, Lcc. lactis ssp.*

Metabolite	Sensorial description	Metabolic pathway	Producing LAB
Ethyl propionate	Winey, grape, and fermented with an eggnog nuance	Esterefication reaction between alcohols and free fatty acids	*Lcb. plantarum*
Ethyl hexanoate	Fruity, pineapple, banana	Esterefication reaction between alcohols and free fatty acids	*Lcc. lactis, Lcc. lactis ssp. lactis, Lcb. rhamnosus, Lcb. paracasei ssp. paracasei, Lcb. plantarum, Lcb. helveticus, Lcb. casei, Leu. lactis, Pediococcus sp*
3-Methyl-1-butanol	Fruity, banana and molasses	Branched-chain amino acids catabolism	*Lcc. Lactis, Lcb. sanfrancisco, Lcc. lactis ssp. lactis*
2-Methyl-propano	Winey	Valine catabolism	*Lcb. helveticus*
Phenyl ethanol	Floral, rose, bready	Phenylalanine catabolism	*S. termophillus, Lcb. rhamnosus*
2-Methyl-butanol	Roasted, winey, fruity	Isoleucine catabolism	*Lcb. brevis, Lcb. fermentum, Lcb. plantarum, Lcb. paracasei ssp. paracasei, Lcb. delbruecki ssp. delbrueckii, E. faecium, S. Termophillus*
1-Hexanol	Green, fruity, apple-skin and oily	3-Keto-hexanoyl-CoA conversion	*Lcb. plantarum*
2,3-Butanediol	Fruity, creamy, buttery	Citrate or aspartate assimilatio	*Lcb. plantarum*
Phenylacetaldehyde	Honey, floral, rose	Phenylalanine catabolism	*O. oeni, Lcb. brevis, Lcb. casei, Lcb. plantarum, Lcc. Lactis*
2-Phenethyl acetate	Floral with a slight yeasty, honey note	Phenylalanine catabolism	*Lcb. plantarum*
Diacetyl	Creamy, buttery	Citrate or aspartate assimilation	*Lcb. plantarum, Lcb. rhamnosus*
Acetoin	Dairy, milky	Citrate or aspartate assimilation	*Lcb. rhamnosus*

The abbreviations of the LAB genera are as follows: *Lactobacillus* = *Lcb.*; *Leuconostoc* = *Leu.*; *Lactococcus* = *Lcc.*; *Streptococcus* = *S.*; *Enterococcus* = *E.*; *Oenococcus* = *O.*; *Pediococcus* = *P.*

2.2.6. Coffee Flavor and Aroma

Coffee flavor precursor compounds have formed naturally in coffee. The precursor compounds are trigonelin, chlorogenic acid, lipids and peptides. However, the presence of fermentation will add to the precursor compounds, namely acids organic, amino acids and reducing sugars. Fermentation will break down carbohydrates into reducing sugars such as glucose and fructose by enzyme activity carbohydrate and pectinase enzymes. Apart from that, there is also decomposition carbohydrates into organic acids such as lactic acid and acetic acid characterized by a decrease in pH and the breakdown of protein into compounds simpler ones like peptides and amino acids. If the precursor to taste incomplete, then the taste and aroma of coffee will not appear when roasting. The compounds formed during roasting are compounds which is so reactive that it plays a role in the next reaction. Reactions this is the formation of pyrazine and non-volatile compounds. Formation the non-volatile compound melanoidin occurs due to the polymerization of sugars and amino ones plays a role in giving the roasted coffee its brown color. The result of the maillard reaction are two groups of compounds, namely volatile and non-volatile compounds. Compound volatiles contribute to aroma and non-volatile c ompounds contribute to taste. Volatile compounds based on studies that have been done generally a compound of the pyrazine group, aldehyde, ketone, phenol, pyridine, pyrole, furan, pyrone, amine, oxazole, thiazole, thiophene, alcohol, benzene, esters, organic acids, sulfur (Towaha et al., 2013). Following is a volatile compound in coffee, as shows in Figure 2.7.

According to research data, furans and their compounds are the largest class after concentration, but pyrazines, aldehydes and ketones are classes with the most commonly known compounds, some of which have strong odorant properties (Moon & Shibamoto, 2009; Piccino et al., 2014; Yang et al., 2016; Steen et al., 2017).

Piccino and co-authors (2014) analysing aromatic composition of coffee identified 107 compounds from which 22 compounds were chosen as potent odorants, for example, 2-furfurylthiol, 3-methylbutanal, 1-ethyl-3-methylbenzene. In Table 5, important aroma, flavour compounds are summarized in specialty coffee.

Ketones and aldehydes are characterized by flower, fruit flavour and pleasant acidity. Fruit and flower notes are not associated with roasted coffee, but the latest research notes regularities that the increase in the quality of coffee increases proportionally volatile compounds with fruit and flower notes (Piccino et al., 2014; Borém et al., 2016; Steen et al., 2017). Almond, cherry flavour is associated with benzaldehyde, strawberry and citrus flavour with 2-phenylacetaldehyde (Piccino et al., 2014). If the fermentation process is not controlled during coffee bean harvesting and the roasting process, the desired aldehyde and ketone compounds can easily convert into spirits and

negatively change the aroma of the coffee composition. Aldehydes and ketones are among the most sensitive compounds and their changes influence sensory evaluation; therefore, the concentration of some aldehydes and ketones is an indicator of the quality of coffee quality.

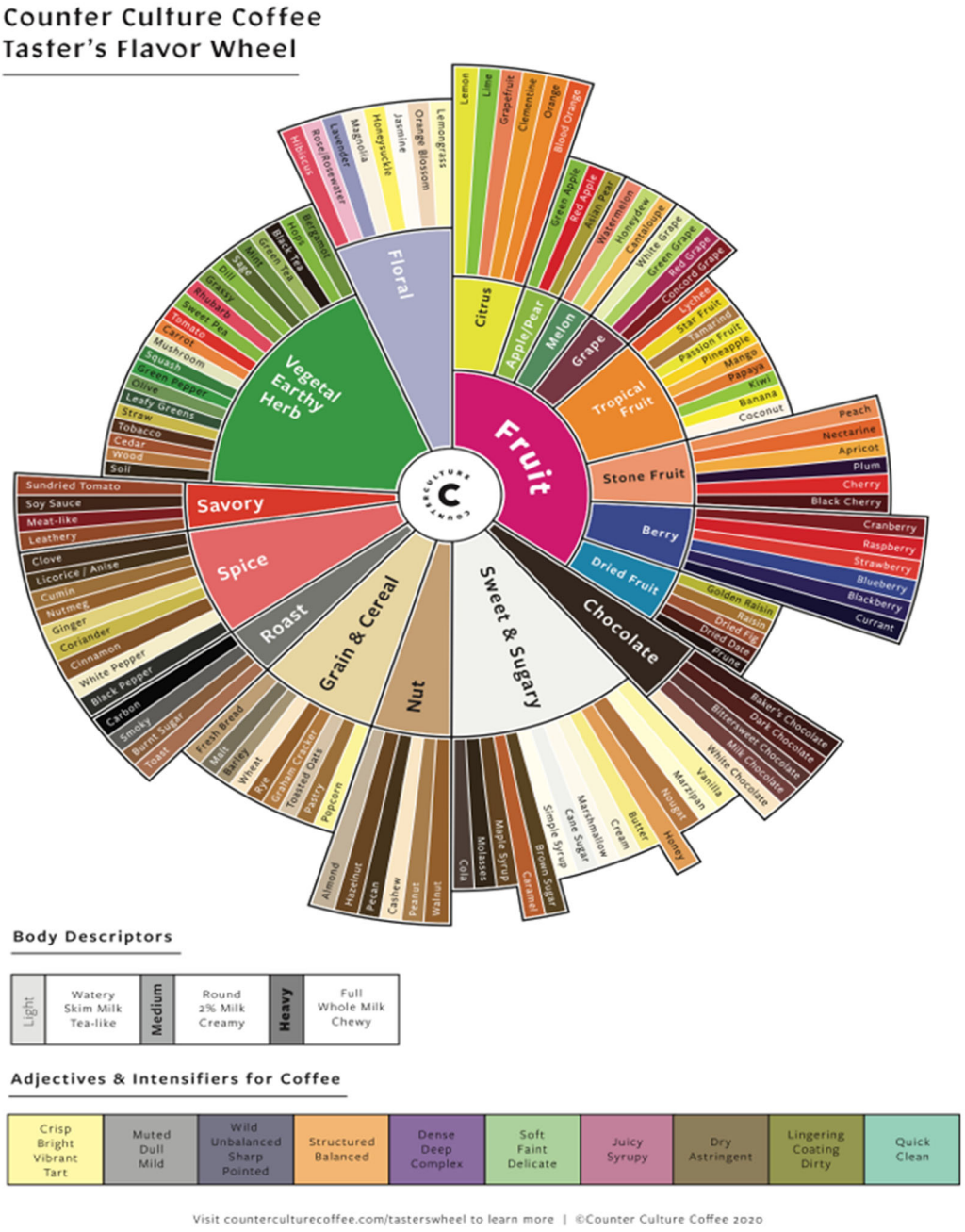


Figure 2.7. Flavor Wheel of Coffee

Table 2.5. Important Aroma, Flavour compounds in Specialty Coffee

Classes	Compounds	Type of Aroma	References
Furans	2-furfurylthiol	roasted coffee	Piccino et al., 2014; Somporn et al., 2011
Aldehydes	(e,e)-nona-2,4-dienal	citrus (fruity)	Piccino et al., 2014
Aldehydes	3-methylbutanal	chocolate	Mestdagh et al., 2014; Piccino et al., 2014
Pyrazines	2-ethyl-3,5-dimethylpyrazine	hazelnut	Mondello et al., 2005; Piccino et al., 2014
Aldehydes	2-phenylacetaldehyde	floral (rose)	Piccino et al., 2014
Pyrazines	2,3-dimethyl-5-ethylpyrazine	hazelnut, cacao	Yang et al., 2016; Piccino et al., 2014
Furanones	4-hydroxy-2,5-dimethyl-3(2h)-furanone	strawberry, caramel	Piccino et al., 2014; Toledo et al., 2016
Alcohols	linalool	floral, fruity	Piccino et al., 2014; Toledo et al., 2016

The sweetness and acidity is highly influenced by organic acid balance – malic, citric and phosphoric acid. Malic and citric acids have positive correlation with sucrose content in coffee (Jham et al., 2007; Borém et al., 2016). Malic acid has apple flavour, but a sensory assessment has often been characterized also with plum, pears and apricot flavour notes and has been associated with a sensory quality, especially, with a pleasant acidity (Borém et al., 2016). Citric acid is known for citric fruit flavour notes (lemon, lime), and phosphoric acid is associated with a grapefruit. Lactic acid and hexanoic acid have buttery, creamy flavour and provide pleasant acidity (Jham et al., 2007; Moon & Shibamoto, 2009). Various research have proven that the amount of chlorogenic acid is lower in high quality roasted coffee than in low-quality roasted coffee (Coelho et al., 2014; Tolessa et al., 2016). Chlorogenic acid in the process of roasting is converted into lactones which give the coffee a pleasant bitter taste (Moreira et al., 2012; Cheong et al., 2013). If the roasting process continues from light – medium roast to medium-dark chlorogenic acid lactones are transformed to phenylindanes which give strong bitter, burning taste (Yang et al., 2016; Tolessa et al., 2016).

However, studies have been found to confirm that it is possible to provide high-quality coffee without losing significant levels of chlorogenic acid. For high quality coffee, it is necessary to ensure that defects are turned off before roasting and chooses a light-medium degree of roasting. The exclusion of defects reduces the removal of unfavourable derivatives of chlorogenic acid, but in light-medium roasted coffee, compounds that highlight pleasant bitterness (Zanin et al., 2016). Oliveira Fassio and co-authors (2016) confirmed that roasted coffee from Catigua cultivar can contain high final sensory score and higher 5-caffeoylquinic acid concentration.

Furans, mostly associated with caramel and a spicy flavour at light roasted coffee and bitter, spicy flavour with increasing roast and serving temperature, for example, furaneol is associeted with sweet caramel, but only in light roasted coffee (Somporn et al., 2011; Cheong et al., 2013; Bressanello et al., 2017). Overall, furans have high odor thresholds, but high furan concentration sets them as important flavours for the sensory quality. Except 2-Furfurylthiol is highly important compound for volatile composition of coffee, because of very low odor thresholds and high concentration in roasted coffee, with chocolate, roasted flavour notes (Moon & Shibamoto, 2009; Piccino et al., 2014; Kim et al., 2018).

Pyrazine compounds are characterized by nutty flavour in roasted coffee and herbal flavour in green coffee. The quality of coffee has a significant effect on the pyrazine compounds, since some of the compounds have a pleasant nutty flavor, but some of the compounds have distinct odor characteristics with a grass, wood flavour that affects the overall sensory rating (Yang et al., 2016; Piccino et al., 2014). Specialty coffee has a distinctly higher amount of pyrazine compounds with positive flavour notes, such as ethyl pyrazine with peppermint flavour, 3-ethyl2,5-dimethyl-5-ethylpyrazine and 2,3-dimethyl-5- ethylparazine with a coconut flavor (Piccino et al., 2014; Steen et al., 2017).

2.3.Material and Method

2.3.1. Material

The materials used were unfermented robusta coffee (Canephora coffee) harvested from Sidomulyo Village, Jember Regency, Indonesia, commercial kefir. The materials used for analysis were NaCl, and alcohol 70% were purchased from Merck, HCl, NaOH, The tools used in this study were automatic fermentor capacity 5 kg (CV. Asmak Kopi, Indonesia), roaster capacity 10 kg (AR, Indonesia), grinder, cup test assessment forms, pH meters, HPLC (GL sciences, Japan), vortex (Hitachi, Japan).

2.3.2. Methods

2.3.2.1.Refermentation of Canephora coffee bean

Unfermented Robusta coffee was sorted from damaged beans and foreign objects. Robusta coffee resulted from sorting as much as 1000 grams was soaked in 2L using distilled water for 2 hours. Wet Robusta coffee beans were drained and added 2% lactose and 1% starter from weight of coffee bean. Re-fermentation was carried out for 0, 6, 12 and 18 hours using in reactor with controlled temperatures 27 ℃, 37 ℃, and 47 ℃, three replicates. The next step was washing robusta coffee beans (*Cafea Canephora*) using water and dried in the sun drying (T: 26-28 ℃; RH: 80-85%) for 2 days until the moisture content became 12%. Complete method described in Figure 2.8 below. Reactor that we used, in Figure 2.9.

2.3.2.2.Sample Preparation for Analysis

Fermented coffee beans are roasted using a temperature of 170-degree Celsius for 9 minutes (medium roasted) to form a flavour and reduce the water content of the coffee. Then roasted coffee was reduced in size using a grinder into coffee powder. The coffee powder produced was then tested cup test (sensory analysis) in the Indonesian coffee and cocoa research centre laboratory in Jember Indonesia by expert panellists and chemical analysis. Flow chart of sensory analysis preparation and chemical analysis can be seen in Figure 2.10.

2.3.2.3.Lactic Acid Bacteria Population

The lactic acid bacteria population were calculated using BAM method (Fardiaz, 1989). The coffee samples (5g) diluted with 45mL sterile distilled water. Dilution was carried out up to 10^{-7}. Dilution results were taken 1mL from the last three dilution levels and poured into a Petridis then added with MRSA and $CaCO_3$. Then, it was incubated at 37 ℃ for 48 hours.

2.3.2.4. pH analysis

Measurement of pH value using pH meter (Muchtadi, 2010). Dry coffee bean after fermentation 0, 6, 12, 18 hours were crushed and dissolution in a ratio of 1:3 ground coffee and distilled water.

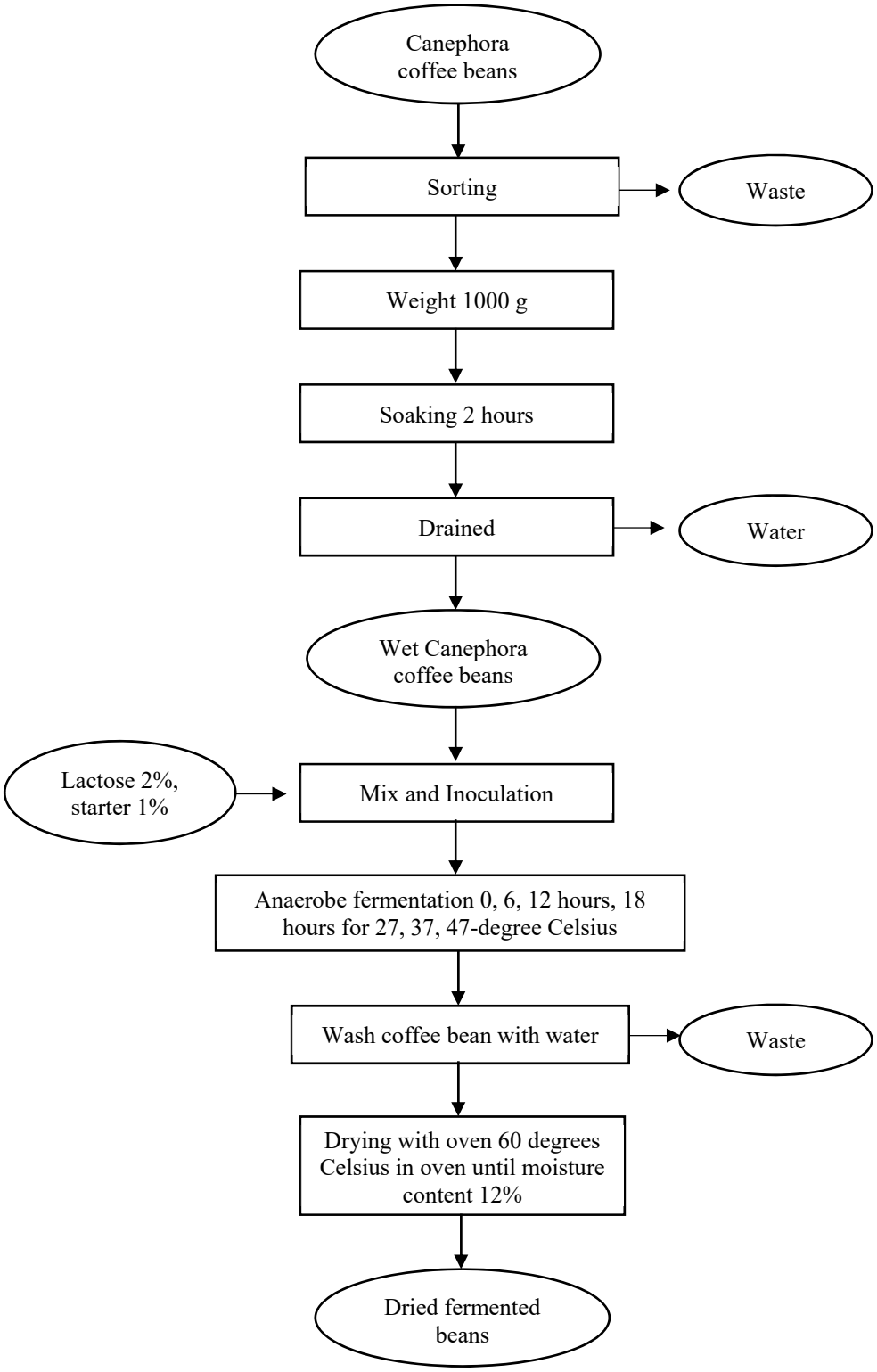
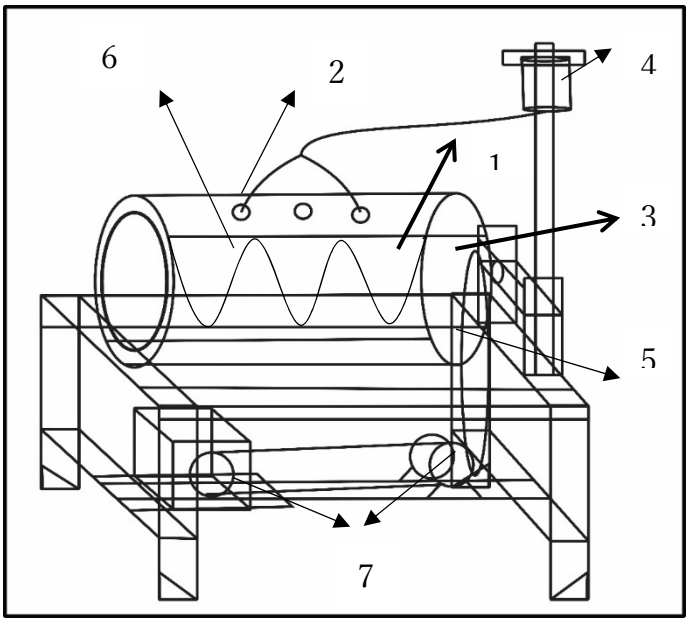


Figure 2.8. Fermentation of Canephora coffee bean

Information:
1. Stainless steel, thick 3 mm
2. Inner diameter 400 mm, outer diameter 550 mm and length 900 mm
3. Capacity of drum 5 kg
4. Velocity of stirrer 12 rpm
5. Power 2HP, 220 Volt

a.



Information of part:
1. Reactor tube
2. Water and starter inlet holes
3. Heater
4. Starter place
5. Motor
6. cylinder type stirrer
7. hinge

b.

Figure 2.9. (a). Automatic Fermentor; (b). Part of fermentor

2.3.2.5.Volatile compound identification

Testing of volatile compounds according to Agresti et al. (2008) conducted with the initial stage is to enter ground coffee (3 g) in the 5 ml vial. Then the sample was heated using a temperature of 70ºC for 10 minutes. Then the SPME needle inserted into the vial and the fibers are directed in the empty cavity above the sample coffee with a temperature of 70ºC for 40 minutes. The GC-MS analysis was carried out with GC-MS Agilent 7890-5975C. Chromatographic separation was performed with an RTX-5MS column internal diameter and Helium as carrier gas at a constant flow of 1.0mL/min. Injector temperature 250 ℃. Ion and interface temperature were 300 ℃ and 275 ℃. The oven initial temperature was 40 ℃ (5 mins), increased to 180 ℃ with 3 ℃/min rate, and then

increased to 250 ℃ (5 mins) with 10 ℃/ min rate. The GC injector was set in split less mode. Volatile compounds identification was based on comparison of their mass spectra and that presented in NIST 2,0 database and confirmed by comparison of their retention indexes with the published references. Linear retention indexes (RI) were calculated using the retention data of linear alkane's solution in n-hexane (Belgis et al., 2017).

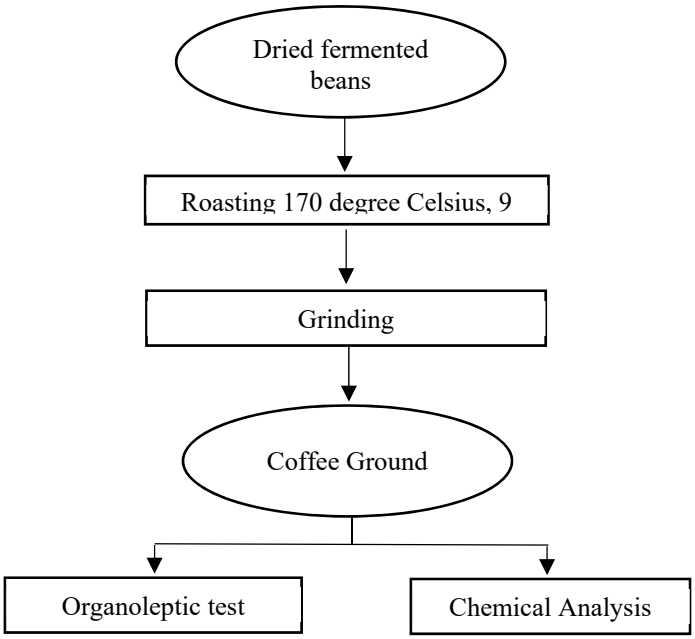


Figure 2.10. Flowchart of preparation sample of sensory and chemical analysis

2.3.2.6.Sensory Analysis

Sensory analysis was performed with cup test using the UCDA method (2010). Each cup contains 10-11 g of roasted coffee. For brewing done with the ratio of coffee and water is 1:10 and the water temperature is 99ºC. The first test what is done is a fragrance test (coffee aroma in dry form). Furthermore, coffee aroma testing is carried out by brewing coffee use hot water and let stand for 5 minutes so that the coffee is extracted optimal. Coffee aroma testing is done by breaking down the layers of the coffee float and smell the aroma. Coffee taste testing was carried out with take 1 teaspoon of coffee and then inhale and taste: flavor, aftertaste, salty / acidity, bitterness / sweetness, balance, clean up, uniform cup, overalls and flaws in taste. Mouthfeel / body testing is done with take 1 steeping spoon of coffee then rub the coffee liquid onto palate and felt.

The description of the scores in the cup test parameters which are divided into 5 categories can be seen as follows:

a. Good : 6,00 – 6,75
b. Very good : 7,00 – 7,75

c. Fine : 8,00 – 8,75

d. Outstanding : 9,00 – 9,75

2.3.2.7. Statistical Analysis

The chemical parameters (pH, C/N ratio, NH_4/NO_3 ratio, fiber, protein, EC) was tested by descriptive method with three repetition and standard deviation.

2.4. Results and Discussion

2.4.1. pH analysis

Testing the pH of the fermented coffee beans aims to determine the acidity level of the coffee beans. The results of coffee bean pH testing can be seen in Fig 2.11. It can be seen that the pH of control coffee beans (inferior Robusta coffee) has the highest pH, which is 6.2. Schwan and Fleet (2015) state that fermentation can cause the pH of coffee beans to decrease due to the breakdown of sugar or caffeine which will be converted into acidic compounds such as lactic, acetic, butyric and propionic acids.

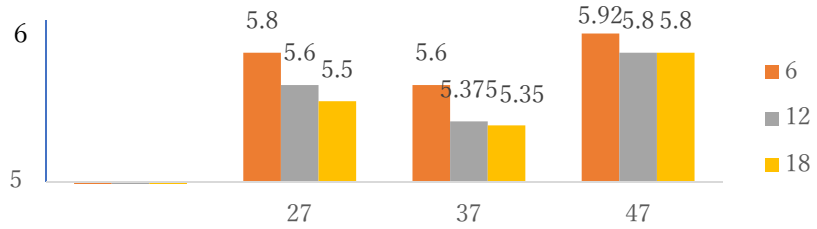


Figure 2.11. pH of Robusta Coffee Beans after Refermentation

During the fermentation process of coffee beans, a starter of kefir milk is added. Kefir is traditional dairy beverage originally from Caucasian and Eastern European regions, produced by the direct addition of kefir grains to milk. Kefir microbial community, which is generally composed of different lactic acid bacteria (LAB), acetic acid bacteria (AAB), and yeast species (Farnworth, 2005). Presence some metabolic products by kefir such as organic acids (lactic acid, acetic acid, butyric acid, propionic acid, and citric acid). This organic acid plays a role in reducing the pH after the coffee refermentation process.

Based on the pH test results, the longer the fermentation time will decrease the pH value of the coffee beans. In fermentation using a temperature of 27ºC. The longer the fermentation time the lower the value. The pH values were 5.8 respectively; 5.6 and 5.52. Decreases in pH values were also reported by Wilujeng and Wikandari (2013), which states that the longer the fermentation takes place, the pH decreases due to amylolytic activity which degrades starch into glucose and subsequently becomes lactic acid. This is confirmed by the opinion of Wardani et al. (2017), which states that during fermentation, Lactic acid bacteria will degrade lactose into glucose and galactose and then become acidic, especially lactic acid and make the pH decrease.

The fermentation temperature has a significant effect on the pH of the coffee beans. Fermentation using a temperature of 37ºC has the lowest pH value compared to seeds coffee is fermented using temperatures of 27 and 47ºC. While fermentation using a temperature of 47ºC has the highest pH value compared to temperature other fermentation. This is in accordance with the research of Wardani et al. (2017) about fermentation of milk using incubation temperatures of 30, 34, 37 and 40ºC. The result showed that the pH value at the fermentation temperature of 40ºC was the highest while fermentation at 37ºC has the lowest pH value. That matter due to the growth of lactic acid bacteria will be directly proportional to acid production and decrease in pH value. For example, at 37ºC fermentation is the optimal temperature for the growth of lactic acid bacteria so that production acid and pH drop is rapid.

The pH value of the coffee beans fermented using a temperature of 47ºC for 6 hours lower than the pH value of coffee beans without fermentation but growth Lactic acid bacteria during that period experienced a decrease in the number of cells. The decrease in pH value can occur because this fermentation is used the source of the inoculum is kefir. According to Otes and Cagindi (2003), kefir is a fermented milk drink that contains a mixture of bacteria and yeast and several types of acids, namely lactic acid and acetic acid. Based on these descriptions can be seen that the temperature and duration of fermentation significant effect on the pH value of fermented robusta coffee beans using a kefir starter.

2.4.2. Lactic Acid Bacteria population

There were 3 types of microorganisms in kefir starter such as LAB, AAB, and yeast (Leite et al., 2013), but the microorganism observed in this study was lactic acid bacteria. Based on previous study by Leite et al (2013), from results identification of Kefir microbial isolates, most microorganism are LAB 60%, AAB 10%, and yeast 11%. The population of lactic acid bacteria during re-fermentation of unfermented Robusta coffee beans using starter can be seen in Figure 2.12.

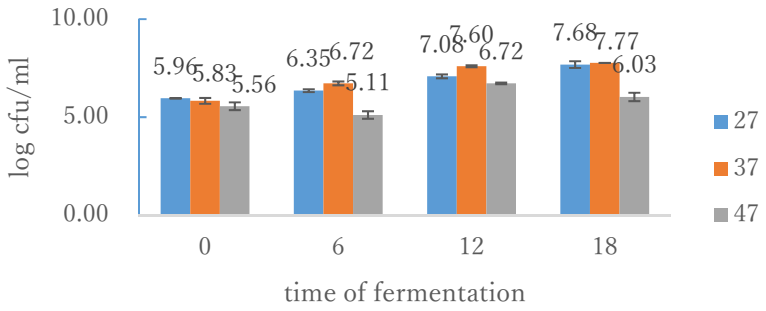


Figure 2.12. Graph of the number of lactic acid bacteria cells in the fermentation of Robusta Coffee beans using a kefir starter at various fermentation temperatures and times.

Based on this graph, it can be seen that fermentation using three different temperature levels affects the growth of lactic acid bacteria. Coffee fermentation at 27 and 37ºC with fermentation time of 0, 6, 12 and 18 hours increased, while at 47ºC there was a decrease in the number of lactic acid bacteria at the 6 and 18 hours. According to Khalid (2011), the optimal temperature for the growth of lactic acid bacteria is 10-45ºC. This is what causes the growth of acid bacteria lactate at a temperature of 47ºC has decreased.

Fermentation at 47ºC resulted in a decrease in the number of lactic acid bacteria from the 0 hour as much as 5.53 log cfu/g after 6 hours of fermentation. 5.08 log cfu/g. The decrease in the number of bacteria was caused because the temperature of 47ºC was not the optimal temperature for the growth of lactic acid bacteria. At this stage the bacteria experiencing a lag phase. The lag phase according to Fardiaz (1989) is the phase that occurs when microorganisms adjust to the substrate and environmental conditions and the number of cells may remain but sometimes decrease. This is in line with the opinion of Rolfe et al. (2012) defined lag phase as the initial period in the life of a bacterial population when a cell acclimates to a new environment before starting the exponential phase.

The number of lactic acid bacteria at 27ºC from 0 to 18 hours always increased with a significant difference. The number of lactic acid bacteria in fermentation using a temperature of 37ºC from 0 to 12 hours also increased significantly. This increase is in accordance with the research reported by Kim et al. (2016), namely fermentation of kefir at a temperature where the number of lactic acid bacteria has increased from the 0 to the 24 hour.

The number of lactic acid bacteria that increased significantly was because the bacterial growth was in the log phase. According to Pelczar and Chan (2007), the characteristics of the log phase in microbial growth are cells dividing at a constant rate, mass doubling at the same rate, constant metabolic activity and a balanced growth state.

The total lactic acid bacteria at 37ºC fermentation with time 12 and 18 were not significantly different, namely 7.59 and 7.76 log cfu / g. At this stage, the bacteria are in a stationary phase. The stationary phase according to Sharah et al. (2015), which is characterized by a constant growth between living and dead bacteria, this is due to reduced nutrients and the formation of metabolic compounds that tend to be toxic to bacteria. According to Mossel et al. (1995), which can cause a stationary phase, namely depletion of nutrient availability, accumulation of toxic metabolic products or a combination of the two so that it will cause some cells to die and some to keep growing. This causes the number of bacteria was constant.

The last stage in the microbial growth phase is the death phase. In this study, the death phase occurred at 45ºC fermentation from the 12 to the 18 hour. At the 12 hour the number of lactic acid

bacteria was 6.72 log cfu / g and at the 18th hour it decreased to 6.0033 log cfu / g and both of them were significantly different. According to Sharah et al. (2015), what is meant by the death phase is the phase in which the number of cells that die is more than the number of new cells. Based on the description above, it can be seen that the temperature and duration of fermentation tend to have a significant effect on bacterial growth Lactic acid in fermentation of the robusta coffee beans using a kefir starter.

2.4.3. Sensory/Cup test

The cup test and volatile compounds were tested on the inferior Robusta coffee beans without fermentation (control) and which had been fermented for 6, 12 and 18 hours at 37ºC. The sample selected for this cup test is based on the results the highest total test for lactic acid bacteria and the lowest pH value. Number of acid bacteria high lactate and low pH value in fermented coffee beans at 37ºC it is thought to have a different taste with Robusta coffee beans random (control). The final score for the Robusta coffee beans can be seen in Figure 2.13.

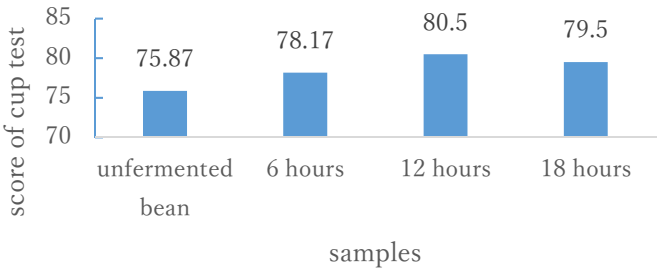


Figure 2.13. Final Score of Robusta coffee on temperature 37ºC

The characteristics assessed on the cup test are aroma, flavour, after taste, acid / salt, bitter / sweet and balance. Refermented beans for 12 hours produce the best score, 80.5. This value is up almost 5 points from the control or sample of unfermented Robusta coffee 75.87. This indicates that refermented Robusta beans have succeeded in entering the specialty coffee category. To be classified as specialty coffee, a coffee needs to obtain a quality score of 80 or higher on a100-point scale from the coffee-tasting process (Specialty Coffee Association of America [SCAA], 2016). From observations by expert panellists, there are several characteristic aromas from each sample, as in table 2.6 below.

Table 2.6. Aroma of Robusta coffee beans at 37ºC

Sample	Characteristic
Control (unfermented coffee)	Chocolaty, bitter
Fermented 6 hours	Chocolaty, bitter, nutty, pappery

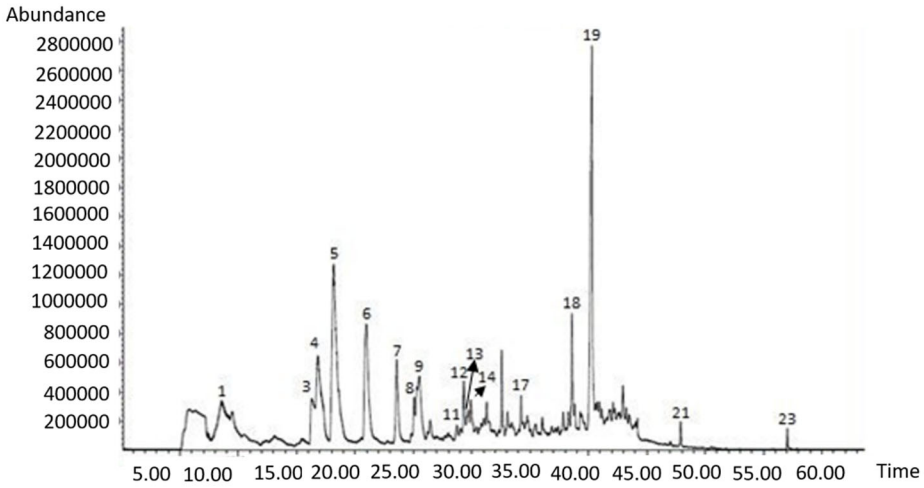
Sample	Characteristic
Fermented 12 hours	Caramelly, soybean, acidy, chocolaty, nutty, pappery
Fermented 18 hours	Spicy, caramelly, acidy, flowery, chocolaty, dried fruit, pappery

Data from cup test by expert panellist

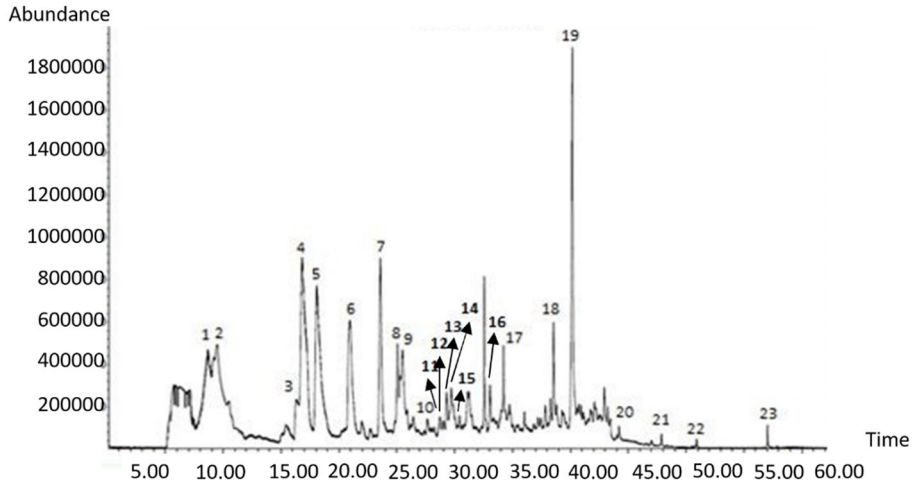
Based on the results of the cup test in Table 2.6, refermented Robusta coffee beans were 18 hours has a characteristic spicy, caramelly, acidy, flowery, chocolaty, dried fruit, and pappery, while for robusta coffee beans the unfermented origin has a characteristic chocolaty and bitter aroma. According to Lee et al. (2015), fermentation will enhance one's aroma pleasant (fruity and acidic) as well as reduced burnt, bitter and aroma woody. This is what causes the longer the fermentation, coffee has a more pleasant aroma so that the results of the fragrance value / aroma are more increasing is directly proportional to the longer fermentation, so it is presumed will be increasingly favoured by consumers.

2.4.4. Volatile Compounds

Volatile compounds have an important role in quality coffee (Sunarharum, 2016). Based on the identification of volatile compounds in unfermented Robusta coffee (control) and refermented using kefir, shows during refermentation an increase in the number of volatile compounds that can be identified. 17 compound of control Robusta coffee beans were identified, while the fermentation treatment with kefir starter at 37ºC shows that it increases with the time of fermentation. Fermentation during 6, 12 and 18 hours were identified 21, 22 and 23 volatile compounds, respectively. Peak image of volatile compounds that have been identified on inferior robusta coffee beans fermented at 37ºC for 6, 12 and 18 hours respectively can be seen in Figure 2.14.



(a).



(b.)

Figure 2.14. (a.) The peak volatile compounds of inferior Robusta coffee beans, fermentation 0 hours; (b.) fermentation 18 hours

From the peak above, we can list volatile compounds in coffee bean that have been identified, in Table 2.7.

Table 2.7. List of Volatile Compounds in Robusta Coffee Bean

No.	Volatile Compounds
1.	Acetic acid
2.	Acetic acid, anhydride with formic acid
3.	Methyl pyrazine
4.	Furfural
5.	2-furanmethanol
6.	2,5-dimethyl pyrazine
7.	5-methyl 2-furancarboxaldehyde

No.	Volatile Compounds
8.	2-furanmethanol, acetate
9.	2-ethyl-6-methyl pyrazine
10.	Benzeneacetaldehyde
11.	1-(1H-pyrrol, 2-yl) ethanone
12.	3-ethyl 2,5 dimethyl pyrazine
13.	2-ethyl-3,5-dimethyl pyrazine
14.	2-methoxy phenol
15.	Nonanal
16.	2-nonenal
17.	1-(2-furanylmethyl) 1H-pyrrole
18.	4-ethyl-2-methoxy phenol
19.	2-methoxy-4vinylphenol
20.	a-furfuryliden-a-furylmethylamine
21.	Butylated hydroxytoluene
22.	Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester
23.	Caffeine

The identified volatile compounds can be grouped into 9 groups, acids, pyrazine, furans, phenols, aldehydes, alcohol, acetate, pyrrole and alkaloids. Based on the nine groups of volatile compounds, there are five groups that have a high percent area, namely acids, alcohol, pyrazine, phenol and aldehyde which can be seen in Figure 2.15. According to Caporaso et al. (2018), the groups of volatile compounds found in Robusta coffee beans are aldehyde, pyrazine, ketone, phenol, acid and heterocyclic N.

From Fig 2.15, some of the volatile compound groups that have increased significantly are aldehydes up to the 12 hour refermentation. The 18-hour refermentation time decreased, but the concentration of aldehyde was high, about 20% when compared to other groups. In addition, acid also experienced a significant increase, while pyrrole increased slightly. This phenomenon is related to the metabolic pathway by microorganisms in starter kefir.

Leite et al (2013) stated that some microorganisms in kefir were dominated by LAB, AAB, and yeast. LAB microorganisms that are abundant in kefir are *Leoconostoc mesentroides* (29%) which are heterofermentative bacteria, and *Lactococcus lactis cremoris* (45%) which are homofermentative bacteria. Both microorganisms in the glycolysis pathway produce metabolites such as lactate, acetate and ethanol, which contribute to sour, vinegar, and fruity flavours. The AAB microorganism that is abundant in kefir is *Acetobacter lovaniensis* (10%) which produces acetic acid metabolite product which also contributes to the sour taste. Another microorganism that is also abundant in kefir is yeast, namely Saccharomyces cerevisiae (11%). The metabolite product of this yeast type is ethanol which contributes to the alcoholic flavour.

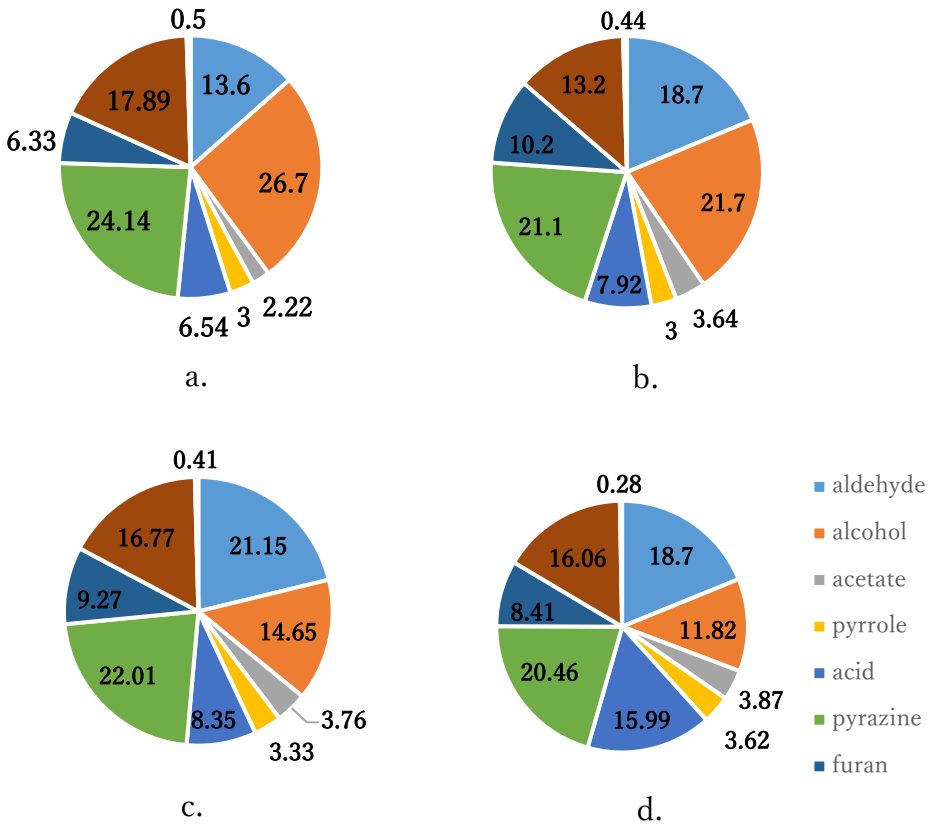


Figure 2.15. Distribution Percentage of Each Volatile Compounds (a) control 0 hour, (b) Refermentation 6 hours, (c) Refermentation 12 hours, (d) Refermentation 18 hours

The extracellular enzymes and organic acids produced from yeast and lactic acid bacteria respectively could potentially lead to the hydrolysis of macromolecules such as carbohydrates, proteins, and polyphenols, generating important aroma precursor such as reducing sugar, amino acids, and chlorogenic acids. Secondary metabolites, produced during refermentation, could also directly or indirectly coffee aroma, such as aldehyde, alcohol (secondary metabolites from branched-chain amino acid metabolism), alcohol (secondary metabolite from sugar metabolism), pyrazines, pyrroles, ketones (secondary metabolite from amino acids metabolism), pyridine, dan nicotinic acid (hydrolysis of polyphenols into chlorogenic acids), ferulic acid, caffeic (hydrolysis of chlorogenic acid into phenolic acids), vanillin (secondary metabolites from phenolic acids metabolism), esters, methyl ketones, alcohols, lactones (secondary metabolites from fatty acid) (Lee et al., 2015).

Changes in each compound associated with fermentation are shown in Figure 2.15. It is indicated by relative evaluation based on the control (t = o). By fermentation, 5-methy2furancaoxyaldehyde was increased about 1.5 times that of control in furans. Phenols overall are decreased. However, in proportion 2 - methoxy - 4 vinylphenol increased about 2 - fold. Furan

compounds identified were 5-methyl 2-furancarboxaldehyde and α-furfuryliden-α-furylmethylamine compounds. 5-methyl 2-furancarboxaldehyde compounds contribute to the burnt aroma, sweet and spicy (Burdock, 2010). These furan compounds were formed by the thermal degeneration of carbohydrates, ascorbic acid, or unsaturated fatty acids during coffee roasting (Sunarharum, 2016). The spicy aroma identified in this study is not only produced by furan group but also affected by the presence of phenol group compounds, namely 2-methoxy-4vinylphenol (Cannon et al., 2010).

The compounds included in the phenol group in this study were 4-ethyl-2-methoxy phenol; 2-methoxy-4vinylphenol; butylated hydroxytoluene and 2-methoxy phenol. Based on the research conducted by Lee et al. (2015), guaiacol (2-methoxy phenol) and p-vinylguaiacol (2-methoxy-4vinylphenol) compounds have increased by the existence yeast fermentation p-vinylguaiacol compounds was formed when enzymatic decarboxylation occurs in ferulic acid compounds while guaiacol is formed due to redox reaction, decarboxylation oxidative and non-oxidative compounds -vinylguaiacol. In the phenol group, there were 2-methoxy-4vinylphenol compounds that contribute to the spicy aroma (Cannon et al., 2010). The area of the 2-methoxy-4vinylphenol compound increases along with the longer fermentation. So, it can be interpreted that the longer the fermentation of the spicy aroma in coffee, the aroma will become stronger.

2-furunmethanol as alcohols decreased about till 60% of control, while Furfural in aldehydes increased by 1.5 times. The compounds included in alcohol group in this study were 2-furanmethanol. According to Caporaso et al. (2018), Robusta coffee generally has 2-furanmethanol compound with high concentrations. These compounds contribute to burnt, sweet, caramel, coffee and bitter aroma in coffee (Zapata et al., 2017). Some volatile compounds included in aldehyde group in this study were furfural compounds; benceneacetaldehyde; nonanal and 2-nonenal. These compounds contribute to sweet, burnt, caramel, nutty, fruity, and fatty aromas (Kivancli and Elmaci, 2015; Burdock, 2010). This aldehyde group was increases along with the length of fermentation. According to Dan et al. (2017), aldehyde was the main volatile compounds found in fermented milk products that can increase volatility.

Benzeneacetaldehyde compounds have an increasing in area along with the length fermentation. This considered as the cause that aldehyde compounds can be produced directly from ethanol by alcohol dehydrogenase activity (Dan et al., 2017). Benzeneacetaldehyde compounds identified in the re-fermentation of Robusta coffee beans contribute to give sweet and fruity aroma (Burdock, 2010). It is considered that the re-fermentation of 18-hour unfermented Robusta coffee beans in commercial kefir have a fruity aroma.

Acetic acid in Acids, 2-furuanmethanol acetate in Acetates and 1-(2-furanylmethyl)1H-pyrrole were increased 1.5-2 times, while Pyrazines were decreased overall. The acetate group can be identified in coffee re-fermentation was 2-furanmethanol acetate. This compound has increased along with the longer fermentation. This acetate 2-furanmethanol compound has the same contribution to the benzeneacetaldehyde compound which can give the characteristics of fruity aroma (Kivacli and Elimaci, 2015).

According to Caporaso et al. (2018), pyrazine compounds which generally identified in Robusta coffee beans were a 2-methyl-pyrazine compound; 2.6-dimethylpyrazine; 2.5-dimethylpyrazine; ethylpyrazine, 2-ethyl-6-methylpyrazine, 2-ethyl-5-methylpyrazine and 3-ethyl-2.5-dimethyl pyrazine. According to Lee et al. (2015), pyrazine compounds are formed from amino acid precursors which occur during caramelization on roasting. Methyl pyrazine compounds; 2.5-dimethyl pyrazine; 2-ethyl-6-methyl pyrazine; 2-ethyl-3.5-dimethyl pyrazine and 3-ethyl 2.5-dimethyl pyrazine contribute in aroma nutty, roasted, cocoa, chocolate and coffee in coffee (Kivancli and Elmaci, 2015).

The pyrrole compounds were can identified in this study were 1-(2-furanylmethyl) 1H-pyrroles and 1-(1H-pyrrol, 2 yl) ethanone. Pyrrole compounds was formed from the amino acids degradation during the roasting process (Poltronieri and Rossi, 2016). These pyrrole compounds contribute to the aroma of nutty, sweet and burnt (Zapata et al., 2017).

Caffeine compounds were identified and belong to the alkaloid class. Based on the area, this compound has decreased due to the breakdown of caffeine compounds into other compounds such as caffeic acid (Lee et al., 2015). According to Burdock (2010), caffeine is a volatile compound that does not contribute to the aroma of coffee compounds. Thus, the decrease of this compound has no effect on the aroma of coffee produced.

CONCLUSION

Totally 17 volatile compounds were identified in unfermented robusta coffee beans. Re-fermentation of coffee bean can enhance the pleasant aroma until 23 compounds in 37°C for 18 hours of fermentation. Some compounds group including of acid, alcohol, aldehyde and acetate groups were contributed to acidy, fruty, nutty and caramelly aroma.

REFERENCES

Agresti, P. D. M., Franca, A. S., Oliveira, L. S., & Augusti, R. 2008. Discrimination between defective and non-defective Brazilian coffee beans by their volatile profile. Food Chemistry, 106(2), 787–796. https://doi.org/10.1016/j.foodchem.2007.06.019

Arya, M., and Rao, L. J. M. 2007. An impression of coffee carbohydrates. Critical Reviews in Food Science and Nutrition, 47, 51-67. https://doi.org/10.1080/10408390600550315

Afriliana A. 2018. A book: Technology of Recent Coffee processing. Jogjakarta, Indonesia: Depublish.

Avallone, S., J. M. Brillouet, B. Guyot, E. Olguin, and J. P. Guiraud. 2002. Involvement of pectolytic microorganisms in coffee fermentation. International Journal of Food Science and Tecnology 37: 191-198.

Belgis, Maria & Wijaya, Christofora & Apriyantono, Anton & Kusbiantoro, Bram & Yuliana, Nancy. 2017. Volatiles and aroma characterization of several lai (Durio kutejensis) and durian (Durio zibethinus) cultivars grown in Indonesia. Scientia Horticulturae. 220. 291-298. DOI: 10.1016/j.scienta.2017.03.041.

Belitz, H. D., Grosch, W., and Schieberle, P. 2009. Food chemistry. (4th ed). Heidelberg: Springer, (chapter 21).

Borém, F.M., Figueiredo, L.P., Ribeiro, F.C., Taveira, J.H.S., Giomo, G.S. & Salva, Tt.J.G. 2016. The relationship between organic acids, sucrose and the quality of specialty coffees. African Journal of Agricultural Research 11(8), 709–717.

Burdock, G. A. 2010. *Fenaroli's Handbook of Flavor Ingredients Sixth Edition.* London: CRC Press.

Cannon, R. J., L. Trinnaman, B. Grainger dan A. Trail. 2010. *The Key Odorants of Coffee from Various Geographical Locations*. Washington DC: American Chemical Society.

Caporaso, N., M. B. Whitworth, C. Cui dan I. D. Fisk. 2018. Variability of Single Bean Coffee Volatile Compounds of Arabica and Robusta Roasted Coffees Analysed by SPME-GC-MS. *Food Research International* 108: 628-640.

Carvalho M, Charles Spence. 2018. The shape of the cup influences aroma, taste, and hedonic judgements of specialty coffee. Food Quality and Preference 68, 315-321, https://doi.org/10.1016/j.foodqual.2018.04.003.

Carvalho, R. D., Breyner, N., Menezes-Garcia, Z., Rodrigues, N. M., Lemos, L., Maioli, T. U., et al. 2018. Secretion of biologically active pancreatitis-associated protein I (PAP) by genetically modified dairy Lactococcus lactis NZ9000 in the prevention of intestinal mucositis. Microb. CellFact.16:27.doi:10.1186/s12934-017-0624-x

Chen, X., Xu, J., Shuai, J., Chen, J., Zhang, Z., and Fang, W. 2007. The S-layer proteins of Lactobacillus crispatus strain ZJ001 is responsible for competitive exclusionagainstEscherichiacoliO157:H7andSalmonellatyphimurium.Int.J.FoodMicrobiol.115,307–312.doi:10.1016/j.ijfoodmicro.2006.11.007

Cheong MW, Tong KH, Ong JJM, Liu SQ, Curran P, Yu B. 2013. Volatile composition and antioxidant capacity of Arabica coffee. Food Res. Int. 51:388-396.

Clarke, R. J. and Macrae, R. (eds.) 1985. Coffee, Volume 1: Chemistry, 1st ed. Elsevier Applied Science, London.

Clifford, M. N. and Willson, K. C. 1985. Coffee - Botany, Biochemistry and Production of Beans and Beverage. Croom Helm, London.

Correa, E.C., Jiménez-Ariza, T., Díaz-Barcos, V., Barreiro, P., Diezma, B., Oteros, Ruiz-Altisent, M. 2014. Advanced characterisation of a coffee fermenting tank by multi-distributed wireless sensors: spatial interpolation and phase space graphs. Food and Bioprocess Technology, 2, 46–54.

Correa-Ascencio, Marisol & Robertson, Ian & Cabrera-Cortés, Oralia & Cabrera-Castro, Rubén & Evershed, Richard. 2014. Pulque production from fermented agave sap as a dietary supplement in Prehispanic Mesoamerica. Proceedings of the National Academy of Sciences of the United States of America. 111. 10.1073/pnas.1408339111.

Dan, T., T. Wang, S. Wu, R. Jin, W. Ren, dan T. Sun. 2017. Profiles of Volatile Flavor Compounds in Milk Fermented with Different Proportional Combinations of *Lactobacillus delbrueckii* subsp. *Bulgaricus* and *Streptococcus thermophilus*. *Molecules* 22: 1633.

Davids K. 2019. Anaerobic Fermentation and Other Palate-Bending Processing Experiments. https://www.coffeereview.com/ [24 November 2020]

De Bruyn, F., Zhang, J. S., Pothakos, V., Torres, J., Lambot, C., Moroni, A. V., etal. 2017.Exploringtheimpactsofpostharvestprocessingonthemicrobiota and metabolite profiles during green coffee bean production. Appl. Environ. Microbiol.83,e2316–e2398.doi:10.1128/AEM.02398-16.

Drider, Djamel & Bekal, Sadjia & Prévost, Hervé. 2004. Genetic organization and expression of citrate permease in lactic acid bacteria. Genetics and molecular research : GMR. 3. 273-281.

Endo, A., and Salminen, S. 2013. Honeybees and beehives are rich sources for fructophilic lactic acid bacteria. Syst. Appl. Microbiol. 36, 444–448. doi: 10.1016/j.syapm.2013.06.002

Endo, A., Tanaka, N., Oikawa, Y., Okada, S., and Dicks, L. 2014. Fructophilic characteristics of Fructobacillus spp. may be due to the absence of an alcohol/acetaldehyde dehydrogenase gene (adhE). Curr. Microbiol. 68, 531–535. doi: 10.1007/s00284-013-0506-3

FAO. 2004. Fermentation of coffee control of operation (p. 10). Roma, Italia: Food and Agricultural Organization.

Fardiaz. S. 1989. A book: Food Microbiologi. Pusat Antar Universitas Pangan dan Gizi. Indonesia: Institut Pertanian Bogor.

Farida, A., E. Ristanti, dan A. C. Kumoro. 2013. Decreasing of Caffeine content and total acid on Robusta coffee using Nopkor MZ-15 in Anaerob Fermentation Technology. Journal of

Chemistry and Industry, 2(3): 70-75.

Fassio, Larissa & Malta, Marcelo & Carvalho, Gladyston & Liska, G.R. & Lima, Priscilla & Pimenta, Carlos. 2016. Sensory Description of Cultivars (Coffea Arabica L.) Resistant to Rust and Its Correlation with Caffeine, Trigonelline, and Chlorogenic Acid Compounds. Beverages, 2, 12. DOI: 10.3390/beverages2010001.

Fauzi .M, et al., 2008. Chemical characteristics of Robusta Coffee, Result of Fermentation use Microflora from Luwak Feces. Indonesia, Berkala Ilmiah Pertanian, 1(1), 21-24. http://repository.unej.ac.id/ [17 Agustus 2020]

Gaibor, Dayana Morales and Wilman Carrillo. 2020. Determination of Caffeine Content in Robusta Roasted Coffee (*Coffea canephora*) by RP-UHPLC-PDA. *Asian Journal of Crop Science, 12: 90-96.* **DOI:** 10.3923/ajcs.2020.90.96

Garcia EF, Luciano WA, Xavier DE, da Costa WCA, de Sousa Oliveira K, Franco OL, de Morais Júnior MA, Lucena BTL, Picão RC, Magnani M, Saarela M and de Souza EL. 2016. Identification of Lactic Acid Bacteria in Fruit Pulp Processing Byproducts and Potential Probiotic Properties of Selected Lactobacillus Strains. Front. Microbiol. 7:1371. doi: 10.3389/fmicb.2016.01371

Ghosh P. 2014. Processing and Drying of Coffee – A Review. International Journal of Engineering Research & Technology, 3 (12), 784-794.

Gilbert G. G. Donders & Sarah Lebeer. 2020. Impact of a lactobacilli-containing gel on vulvovaginal candidosis and the vaginal microbiome. Scientific report, nature research, 10: 7976 https://doi.org/10.1038/s41598-020-64705-x

Gonzalez-Rios, O., Suarez-Quiroz, M. L., Boulanger, R., Barel, M., Guyot, B., Guiraud, J.P. 2007. Impact of "ecological" post-harvest processing on the volatile fractions of coffee beans: I. Green coffee. Journal of food Composition and Analysis, 20, 289-296.

Gudi P. 2017. Carbonic Maseration (a unique way of coffee processing). Journal of Applied Microbiology 128, 209-224.

Hanifah, N. dan D. Kurniawati. 2013. Effects of Alkali solution and Yeast on Acid, Caffeine, and lipid concentration on Fermented Coffee Processing. Journal of Chemstry Technology and Industry, 2(2): 162-168.

Hoffman James. 2014. 2nd book edition: The World Atlas of Coffee: From Beans to Brewing - Coffees Explored, Explained and Enjoyed. Mitchell Beazley, England

Holzapfel, W.H., Wood, B.J.B., 2014. Lactic Acid Bacteria: Biodiversity and Taxonomy. John Wiley & Sons.

ICCRI. 2008. Coffee Processing. PT Riset Perkebunan Nusantara, Indonesian Coffee and Cacao Research Institute. http://iccri.net/pengolahan-kopi/[24 November 2019]

ICO. 2020. Coffee Market Report: September 2020 coffee exports rise. http://www.ico.org/ [25 November 2020]

Illy, A. and Viani, R. 1995. Espresso Coffee: The Chemistry of Quality Londres, UK: Academic Press.

Jackels, S. C. and C. H. Jackels. 2005. Characterization of the coffee mucilage fermentation process using chemical indicator: a field study in Nicaragua. Journal of Food Science 70 (5): 321-325

Jham, Gulab & Fernandes, Sergio & Garcia, Cleverson. 2007. Comparison of GC and HPLC for quantification of organic acids in two jaboticaba (Myrciaria) fruit varieties. Química Nova. 30, 1529-1534. DOI: 10.1590/S0100-40422007000700006.

Kaseleht, K. 2012. Identification of Aroma Compounds in Food Using SPME- GC/MS and GC-Olfactometry. *Thesis.* TUT Press.

Khalid, K. 2011. An Overview of Lactic Acid Bacteria. *International Journal of Biosciences (IJB)* 1(3): 1-13.

Kim, KW., Won, Y.L. & Ogawa, Y. 2018. Associations between Blood Levels of Polychlorinated Dibenzo-p-dioxins/Furans and Polychlorinated Biphenyls with Immune Systems. *Toxicol. Environ. Health Sci.* **10,** 17–25. https://doi.org/10.1007/s13530-018-0342-8

Kivancli, J. dan Y. Elmaci. 2015. Characterization of Turkish-style Boiled Coffee Aroma by GC/MS and Descriptive Analysis Techniques. *International Journal of Food Properties.*

Lee, Liang & Cheong, Mun Wai & Curran, Philip & Yu, Bin & Liu, Shao. 2015. Coffee fermentation and flavor–An intricate and delicate relationship. Food Chemistry. 185. DOI: 10.1016/j.foodchem.2015.03.124.

Leite AM, Leite DC, Del Aguila EM, Alvares TS, Peixoto RS, Miguel MA, Silva JT, Paschoalin VM. 2013. Microbiological and chemical characteristics of Brazilian kefir during fermentation and storage processes. J Dairy Sci. 96(7):4149-4159. http://doi: 10.3168/jds.2012-6263.

Leong, Kun-Hon & Chen, Yi-Sheng & Pan, Shwu-Fen & Chen, Jen-Jye & Wu, Hui-Chung & Chang, Yu-Chung & Yanagida, Fujitoshi. 2014. Diversity of Lactic Acid Bacteria Associated with Fresh Coffee Cherries in Taiwan. Current microbiology, 68. DOI: 10.1007/s00284-013-0495-2.

Lin, C. C. 2010. Approach of improving coffee industry in Taiwan promote quality of coffee bean by fermentation. The Journal of International Management Studies 5 (1): 154-159.

Masoud, Wafa & Kaltoft, Christa. 2006. The effects of yeasts involved in the fermentation of Coffea Arabica in East Africa on growth and ochratoxin A (OTA) production by *Aspergillus ochraceus.* International journal of food microbiology. 106. 229-34. 10.1016/j.ijfoodmicro.2005.06.015.

Mayo, Baltasar & Aleksandrzak-Piekarczyk, Tamara & Fernández, María & Kowalczyk, Magdalena & Álvarez-Martín, Pablo & Bardowski, Jacek. 2010. Updates in the Metabolism of Lactic Acid Bacteria. 10.1002/9780813820866.ch1.

Minamisawa, M., Yoshida, S., and Takai, N. 2004. Determination of biologically active substances in roasted coffee using a diode-array HPLC system. Analytical Sciences, 20, 325-328.

Mondello, L., Costa, F., Tranchida, P.Q., Dugo, P., Presti, M.L., Festa, S., Fazio, A., & Dugo, G. 2005. Reliable characterization of coffee bean aroma profiles by automated headspace solid phase microextraction-gas chromatography-mass spectrometry with the support of a dual-filter mass spectra library. Journal of Separation Science, 28, 1101–1109.

Moon, J.K., & Shibamoto, T. 2009. Role of roasting conditions in the profile of volatile flavor chemicals formed from coffee beans. Journal of Agricultural and Food Chemistry, 57(13), 5823–5831. DOI: 10.1021/ jf901136e.

Moreira MEC, Pereira RGFA, Dias DF, Gontijo VS, Vilela FC, Moraes GOI, Giusti-Paiva A, Santos MH. 2013. Anti-inflammatory effect of aqueous extracts of roasted and green Coffea arabica L. J. Funct. Foods 5:466-474.

Mossel, D. A. A., J. E. L. Corry, C. B. Struijk dan R. M. Baird. 1995. *Essentials of The Microbiology of Foods: a Textbook for Advanced Studies*. England: John Wiley and Sons.

Muchtadi, T. R., Sugino dan F. Ayustaningwarno. 2010. Food Science. Bandung Indonesia: Alfabeta.

Multon, J.L., Poisson, B., and Cachaginer, M. 197). Evolution de plusieurs caratéristiquers d'un café Arabica au cours d'un stockage experimental effectuéà 5 humidités relatives at 4 températures différentes. Proceedings of the 6th ASIC Colloquium, 268–277.

Nava, Ernesto Favela-Torres and Gerardo Saucedo-Castaneda. 2011. Effect of Mixing on the Solid-State Fermentation of Coffee Pulp with Aspergillus tamarii. Food Technol. Biotechnol. 49 (3) 391–395.

Neto C, Gilberto V. de Melo Pereira, Ana M.O. Finco, Luiz A.J. Letti, Bruno J.G. da Silva, Luciana P.S. Vandenberghe, Carlos R. Soccol. 2018. Efficient coffee beans mucilage layer removal using lactic acid fermentation in a stirred-tank bioreactor: Kinetic, metabolic and sensorial studies. Food Bioscience, 26, 80-87. https://doi.org/10.1016/j.fbio.2018.10.005.

Otes, S., dan O. Cagindi. 2003. Kefir: a Probiotic Dairy-Composition, Nutritional and Therapeutic Aspects. *Pakistan Journal of Nutrition* 2(2): 54-59.

Otes, S., dan O. Cagindi. 2003. Kefir: a Probiotic Dairy-Composition, Nutritional and Therapeutic Aspects. *Pakistan Journal of Nutrition* 2(2): 54-59.

Pelczar, M. J. dan E. C. S. Chan. 2007. *Basic of Microbiology volume 1*. Jakarta: UI Press.

Pereira GV, Beux M, Pagnoncelli MG, Soccol VT, Rodrigues C, Soccol CR. 2016. Isolation, selection and evaluation of antagonistic yeasts and lactic acid bacteria against ochratoxigenic fungus *Aspergillus westerdijkiae* on coffee beans. Lett Appl Microbiol. 62(1):96-101. doi: 10.1111/lam.12520.

Pereira, O. G., Rocha, K. D., and Ferreira, C. L. D. L. F. 2016. Chemical composition, characterization, and population of microorganisms on elephantgrass "Cameroon" (Pennisetum purpureum, Schum) and its silages. R.Bras.Zootec.36,1742–1750.doi:10.1590/S1516-35982007000800006

Pereira, R.; Park, C. S.; Park. Y. Mechanisms of action of coffee bioactive components on lipid metabolism. 2019. Food Science and Biotechnology. http://doi.org/10.1007/s10068-019-300662-0

Piccino, S., Boulanger, R., Descroix, F., & Sing, A.S.C. 2014. Aromatic composition and potent odorants of the "specialty coffee" brew "Bourbon Pointu" correlated to its three trade classifications. Food Research International, 61, 264–271. DOI: 10.1016/j.foodres.2013.07.034.

Poltronieri, P. Dan F. Rossi. 2016. Review Challenges in Specialty Coffee Processing and Quality Assurance. *Challenges* 7: 19.

Rahmanullah A. 2017. Indonesia coffee annual report. Https://gain.fas.usda.gov/.../Coffee%20Annual_Jakarta_Indonesia_5-152017.pdf [5 august 2018]

Rodrigues, Carla & Maia, Rodrigo & Ribeirinho, Miguel & Hildebrandt, Peter & Gautz, Loren & Prohaska, Thomas & Máguas, Cristina. 2013. Coffee. Comprehensive Analytical Chemistry. 60, 573-598. DOI: 10.1016/B978-0-444-59562-1.00022-0.

Rolfe, M. D., C. J. Rice, S. Lucchini, C. Pin, A. Thompson, A. D. S. Cameron, M. Alston, M. F. Stringer, R. P. Betts, J. Baranyi. M. W. Peck dan J. C. D. Hinton. 2012. Lag Phase is A Distict Growth Phase That Prepares Bacteria for Exponential Growth and Involves Transient Metal Accumulation. *Journal of Bacteriology* 194(3): 686.

Sánchez, B., Arias, S., Chaignepain, S., Denayrolles, M., Schmitter, J. M., Bressollier, P. 2009. Identification of surface proteins involved in the adhesion of a probiotic Bacillus cereus strain to mucin and fibronectin. Microbiology 155, 1708–1716. doi: 10.1099/mic.0. 025288-0

Schwan, R.F. and Fleet, G.H. 2015. Cocoa and Coffee Fermentations. Boca Raton, FL: CRC Press, Taylor & Francis Group.

Schwan, Rosane & Pereira, Gilberto & Fleet, G.H. 2014. Microbial activities during cocoa fermentation. Cocoa and Coffee Fermentations. 129-192.

Sharah, A., R. Karnila dan Desmelati. 2015. Making Growth Curves for Lactic Acid Bacteria Isolated from Bloated Peda Fish (*Rastrelliger* sp.).

Silva, Cristina & Vilela, Danielle & Cordeiro, Cecilia & Duarte, Whasley & Dias, Disney & Schwan, Rosane. 2013. Evaluation of a potential starter culture for enhance quality of coffee fermentation. World journal of microbiology & biotechnology. 29(2), 32-38. DOI: 10.1007/s11274-012-1175-2.

Sinaga A. 2018. Fermentation Process of Arabika Lintong Nihuta: Effect of Variation kind of container and time on Quality Coffee. A thesis, University of Sumatra Utara, Indonesia. https://repositori.usu.ac.id/handle/123456789/9415.

Singh, V., & Singh, G. 2013. Coffee: process technology and health benefits. International Journal of Innovative Research & Studies, 2, 153–160.

Smit, Gerrit & Smit, Bart & Engels, Wim. 2005. Flavour formation by lactic acid bacteria and biochemical flavour profiling of cheese products. FEMS microbiology reviews, 29, 591-610. 10.1016/j.femsre.2005.04.002.

Soenaryo dan Situmorang. 1978. Chocolate Processing. Indonesia, Balai Penelitian Perkebunan Bogor. Pp 32.

Somporn, Chanyarin & Kamtuo, Amnouy & Theerakulpisut, Piyada & Siriamornpun, Sirithon. 2011. Effects of roasting degree on radical scavenging activity, phenolics and volatile compounds of Arabica coffee beans (Coffea arabica L. cv. Catimor). International Journal of Food Science & Technology, 46, 2287 - 2296. DOI: 10.1111/j.1365-2621.2011.02748.x.

Steen, I., Waehrens, S.S., Petersen, M.A., Münchow, M., & Bredie, W.L.P. 2017. Influence of serving temperature on flavour perception and release of Bourbon Caturra coffee. Food Chemistry, 219, 61–68. DOI: 10.1016/j.foodchem.2016.09.113.

Sulistyowati dan Sumartona. 2002. Methode of Flavor Coffee Analysis. Jember, Indonesia: Pusat Penelitian Kopi dan Kakao. pp 19

Sunarharum, W. B. 2016. The Compositional Basis of Coffee Flavour. *Thesis*. Australia. The University of Queensland.

Tika, Ni Made Pujani, I Gusti Ayu Tri Agustiana. 2017. Caffein content in coffee with fermentation using microorganism isolated from Luwak Feces. National seminar Innovative research, 839-846. http://digilib.mercubuana.ac.id/

Toledo, Paulo & Pezza, Leonardo & Redigolo Pezza, Helena & Toci, Aline. 2016. Relationship between the different aspects related to coffee quality and their volatile compounds: Volatile compounds and coffee quality. Comprehensive Reviews in Food Science and Food Safety. 15, 1-15. DOI: 10.1111/1541-4337.12205.

Tolessa, K., D'heer, J., Duchateau, L. and Boeckx, P. 2017. Influence of growing altitude, shade and harvest period on quality and biochemical composition of Ethiopian specialty coffee. Journal of the Science of Food and Agriculture 97(9): 2849-2857.

Towaha J, Anggraini DA, Rubiyo. 2013. Variation of cacao quality and diversification product in some fermentation: Case study in Tabanan Bali Indonesia. Pelita Perkebunan, 28 (3), 166-183.

Uganda Coffee Development Authority - UCDA. Robusta Cupping Protocol. 2010. https://ugandacoffee.go.ug/sites/

Usman, A. Suprihadi, and E. Kusdiyantini. 2015. Fermentation of Robusta Coffee (Coffea canephora) use Isolate Lactic Acid Bacteria from luwak feces with treatment time of incubation, *Jurnal Academika Biology*, 4(3), 31-40. https://ejournal3.undip.ac.id/

Vermeulen M.G, Gänzle R.F. Vogel. 2006. Glutamine deamidation by cereal-associated lactic acid bacteria. Journal of Applied Microbiology 103 (4), 1197-1205. DOI: 10.1111/j.1365-2672.2007.03333.x

Wang, S., Dong, Z., Li, J., Chen, L., and Shao, T. 2019. Pediococcus acidilactici strains as silage inoculants for improving the fermentation quality, nutritive value and in vitro ruminal digestibility in different forages. J. Appl. Microbiol. 126,424–434.doi:10.1111/jam.14146

Wardani, S. K., M. N. Cahyanto, E. S. Rahayu dan T. Utami. 2017. The Effect of Inoculum Size and Incubation Temperature on Cell Growth, Acid Production and Curd Formation during Milk Fermentation by *Lactobacillus plantarum* Dad 13. *International Food Research Journal* 24(3): 921-926.

Widyotomo, S., & Yusianto. 2013. Optimation of fermentation process on Arabica Coffee in Controlled Fermentor. Pelita Perkebunan, 29, 53–68.

Wilujeng, A. A. T. dan P. R. Wikandari. 2013. Effect of Fermentation time Arabica Coffee (*Coffea arabica*) with Lactic Acid Bacteria *Lactobacillus plantarum* B1765 on Quality Product. *Journal of Chemistry* 2(3): 1-10.

Wintgens, J. 2008. Factors Influencing the Quality of Green Coffee. In book: Coffee: Growing, Processing, Sustainable Production: A Guidebook for Growers, Processors, Traders, and Researchers. DOI: 10.1002/9783527619627.ch29.

Yang F, Luo L, Zhu ZD, Zhou X, Wang Y, Xue J, et al. 2017. Chlorogenic acid inhibits liver fibrosis by blocking the miR-21-regulated TGF-β1/Smad7 signaling pathway in vitro and in vivo. Front Pharmacol. 8, 929.

Yang, N., Liu, C., Liu, X., Degn, T.K., Munchow, M., & Fisk, I. 2016. Determination of volatile marker compounds of common coffee roast defects. Food Chemistry, 211, 206–214. DOI: 10.1016/j. foodchem.2016.04.124.

Yusianto and Nugroho. 2014. Physical and Flavor Profiles of Arabica Coffee as Affected by Cherry Storage before Pulping. Pelita Perkebunan (Coffee and Cocoa Research Journal), 30(2), 137-158. http://DOI. 30. 10.22302/iccri.jur.pelitaperkebunan.v30i2.7.

Zanin, Rodolfo & Corso, Marinês & Kitzberger, Cíntia & Scholz, Maria Brigida & Benassi, Marta. 2016. Good cup quality roasted coffees show wide variation in chlorogenic acids content. LWT - Food Science and Technology, 74. DOI: 10.1016/j.lwt.2016.08.012.

Zapata, J., V. Londono, M. Naranjo., J. Osorio, C. Lopez dan M. Quintero. 2018. Characterization of Aroma Compounds Present in An Industrial Recovery Concentrate of Coffee Flavour. *Journal of Food* 16(1): 367-372.

Zhang T, Yanchao Yuan, Yu Zhan, Xinzhe Cao, Chunying Liu, Yuxi Zhang and Shupeng Gai1. 2019. Metabolomics analysis reveals Embden Meyerhof Parnas pathway activation and

flavonoids accumulation during dormancy transition in tree peony. Plant Biology, 20, 484. https://doi.org/10.1186/s12870-020-02692-x

CHAPTER 3

Composting of Spent Coffee Grounds

Chapter 3

3.1.Background Research

Around 9 million tons of spent coffee grounds are produced in the world (Santos, 2017). Coffee is the favorite beverage in the world; therefore, this number always increases every year (Murthy and Naidu, 2012). Consumption per ton of coffee beans can produce 650 kg of coffee grounds (Murthy and Naidu, 2012). An estimated percentage of around 90% of the brewed coffee ends up though in the form of Spent Coffee Grounds (SCG). SCG is characterized by high organic content, 38% hemicelluloses and 9% cellulose, and protein 14%. (Murthy and Naidu, 2012). SCG still contains phenol compounds, tannins, and caffeine (Leifa et al., 2000). This compound is toxic to nature and is a cause of environmental pollution (Cruz et al., 2014). Therefore, it is necessary to treat spent coffee grounds to reduce the toxic component that is by composting. Composting is a simple and efficient way to convert waste into stable, non-toxic, and has good nutrition for soil and plants (Ros et al., 2006b). It generally equipped with heating and agitating devices that promote biodegradation of organic materials by microorganisms under neutral to alkaline conditions at moderate to high temperatures (40–60°C) (Beffa et al., 1998; Insam et al., 2002; Kimura et al., 2000).

In this study, composting of coffee grounds was added by the addition of some fungi as starter culture with temperature control. The starter is an additional material used in the early stages of the process fermentation. Starter is a culture of certain microbes that are grown inside substrate or medium for specific process purposes (Kusumaningati et al., 2013). The requirements for the fermentation starter are pure, superior, stable and not pathogenic. According to Utama et al. (2013) requirements for fermentation starters are safe to use and capable inhibits pathogenic bacteria.

The combination of some microorganism and growth optimization with temperature control is expected to further maximize the production of enzymes and the process of material degradation so as to accelerate the composting process. Fungi are the main decomposers in soil ecosystems (Irawan et al., 2014). In addition, fungi are also able to decompose very well lignocellulose, protein and amino acid. The types of fungi used in this study were *Aspergillus sp* and *Penicillium sp*. it was isolated from fertile soil. Fungal activity of combination *Aspergillus sp* and *Penicillium sp* can also speed up the composting process. Starter of this fungus can improve the biological, physical, and chemical properties of the soil, therefore that plants can grow better and are more resistant to pathogens (Murbandono, 2003).

According to Subowo & Corazon (2010) and Subowo (2015) that *Aspergillus spp.* and

Penicillium spp. has the ability to break down lignin, as well. *Penicillium spp.* and *Aspergillus spp.* is a type of fungus that can grow on media containing lignin (Adlini, 2014). *Penicillium spp.* even can increase plant growth in peat soils because they help provide the elements nutrients for plants by degrading the organic matter (including lignin compounds) in peat soils (Yuleli, 2009). Both of these genus fungi known to have a lignin degradation ability quite good compared to other fungus genera which has also been proven in other studies (Subowo & Corazon, 2010).

Penicillium sp and *Aspergillus sp* are soft rot fungi. Soft-rot fungi are mostly ascomycete fungi that can degrade polysaccharides in the surface layers of plants (Martina et al., 2018). Gupta (2015) and Gupta et al. (2016) mention that soft rot fungi are no doubt the most efficient fungi to degrade lignin in mixed microbial population. Besides that, the adaptation of soft rot fungi in various temperature, different pH, and limited oxygen is higher than other fungi (Madadi M, 2017).

However, still little is known about the degradation mechanism of lignocellulose by soft rot fungi (Martina et al., 2018). Therefore, this study uses soft rot fungi as starter and learn this effect on quality of compost produced. This is because even though the degradation capability is low when compared to white rot and brown rot function as mentioned above in the previous work, soft rot fungi also have good degradation capability. In addition, because compost is one of the best places for the growth of soft rot fungi, apart from soils, piles of woodchip, and straw (Daniel, 1998).

The addition of starter in the composting process has been widely studied by researchers. However, the use of specific combination types of fungi starter with temperature control for SCG composting is still very little studied. Yamane et al (2014) studied the use of coffee grounds directly on plant growth, the results of which took a long time for the benefits of coffee grounds to function positively for the soil and plants. The difference in the use of fresh and composted spent coffee ground is also studied and composted SCG is better for plants (Gomes, 2013). SCG compost using solid state fermentation was also studied by Echeverria et al (2016). Because of the lack of reference composting of SCG using soft rot fungi with temperature control, therefore in this study studied the composting of coffee grounds using fungi especially by *Penicillium sp* and *Aspergillus sp.*

Starter using fungi is a very promising method for increasing agricultural production, as well as reducing the release of chemical pesticides into the environment. This is because they are able to change and release many nutrients that play an important role in the nutrition cycle, and maintain vegetation (Joshi, 2015). In addition, in this study also added cow dung and chicken manure to supply extra carbon for microbial activity, balancing C / N ratio, and providing most of the nutrients, including nitrogen (N), phosphorus (P), and potassium (K).

Composting method of this study is aerobic static batch composting with temperature control. Because can be generated in a short period of time (Zeng et al., 2012; Gill et al., 2014). Temperature

control is done to maximize the growth of microorganisms, especially *Penicillium sp,* and *Aspergillus sp* during the composting process. This is because temperature is one of the most important environmental factors in the composting process. The objective of this study was to understand the effect of using combination fungi with temperature control in SCG composting.

3.2.**Literary Review**

3.2.1. Spent Coffee Grounds (SCGs)

Spent coffee ground (SCG) is the waste that is accumulated after coffee consumption, or it can be described as a by-product of brewing process. Special attention is given to the accumulation of this food production by-product due to high coffee consumption worldwide. This statement is supported by the facts: in 2016, 7.2 million tons of all coffee forms was exported, and from 2000 to 2012, global green coffee production increased by 17% (Campos et al., 2015); in total, 0.91 g wasted grounds per gram of coffee is produced. Around 6 million tons of spent coffee grounds are produced each year (Zhang et al., 2017). Consequently, these facts have made many research teams focus on the usage of spent coffee grounds (Hardgrove et al., 2016).

On the other hand, the release of spent coffee grounds to the environment due to increasing coffee consumption causes environmental contamination due to the fact that its decomposition consumes large quantities of oxygen (Hardgrove et al., 2016). All waste disposal issues represent big economic and environmental problems (Lessa et al., 2018). SCG utilization and management represent a big problem due to the presence of phenols, caffeine, and tannins, which are highly toxic for many life processes. The study showed that the toxicity of SCG could be reduced by warm treatment, microbial biodegradation, and aerobic fermentation (Hao et al., 2018; Caetano et al., 2012; Murthy et al., 2012; Gomez et al., 2015; and Calixto et al., 2011).

SCG is most often part of municipal solid waste, so it can be incinerated or landfilled (Deligiannis, 2011). SCGs, on average, contain 45.3% (w/w, dry weight) of polysaccharides (mannose, galactose, glucose, and arabinose) bound to cellulose and hemicellulose complexes (Musatto et al., 2011; Musatto et al., 2011). Mannans are the major component of polysaccharides in SCGs (Musatto et al., 2011). There are almost no reducing sugars present in coffee (Pasin et al.,

2011). The main phenol presented in coffee is chlorogenic acid (CGA) and accounts for up to 14% (dry matter basis) (Farah et al., 2006). Chemical content of SCG in Figure 3.1.

Figure 3.1. Chemical content of SCG (Saratale et al., 2020)

Moreover, SCG has considerable amount of nutrients such as nitrogen (ranging from 1.2 to 2.3%), phosphorus (ranging from 0.02 to 0.5%), and potassium (averaged in 0.35%) contents (Mussatto et al. 2011a; Cruz et al. 2012) suggesting its application on agriculture sector as fertilizer or as soil conditioner (Kondamudi et al. 2008; Cervera-Mata et al. 2017, 2019). Adding SCG to the soil can have conclusive effects on environment due to the carbon fixation into the soil (Cervera-Mata et al. 2017).

The agronomic potential of SCG still remains unclear despite the fact that the high concentration of nutrients and carbon/nitrogen ratio of SCG could increase soil fertility and plant growth (Cervera-Mata et al. 2017). Hardgrove and Livesley (2016) reported that when SCG applied directly to soils in urban farming, it reduced significantly the plant growth, although other scientists have outlined an increased rate of growth when SCG used in smaller quantities (Cruz et al. 2012). Such limitation is attributed to the existence of caffeine (approximately 0.2%) in espresso SCG, along with tannins and chlorogenic acids, which may enforce certain toxic effects on soil microbiota and crops (Batish et al. 2008; Yamane et al. 2014). Low et al. (2015) noted that composted SCG should be applied to soil rather than fresh, to obtain higher soil mineralization and boost nutrient absorption by the cultivated plants. Cruz et al. (2014) showed that the addition of SCG through composting for cultivating lettuce was a valuable source of bioactive components, demonstrating the potential of the antioxidant pool and vegetable quality produced. Recently it has been shown the partially peat replacement into growing media by using SCG compost for potted plant production; yet, this was species related (Ronga et al. 2016). Composted SCG material could provide sufficient

nutrients and supported the plant growth in potted plants and could possibly acted as peat replacement in a growing media specifically devoted to agricultural seedlings (Ceglie et al. 2015).

3.2.2. Composting

Composting is the transformations of raw organic materials into biologically stable, humic substances suitable for a variety of soils and plant uses. Essentially, composting is controlled decomposition, the natural breakdown process that occurs when organic residue comes in contact with soil (Rynk, 1992). Since composting is a microbially mediated process, providing the proper environmental conditions for microbes to decompose raw organic materials is crucial for success (Fig 3.2).

The three most important factors for making good compost are the chemical makeup of the raw ingredients or feed stocks (quality and quantity of carbon and minerals, pH), the physical size and shape of the feed stocks and the porosity of the pile, and the population of organisms involved in the composting process (macro fauna and mesofauna; microorganisms including bacteria, actinomycetes, fungi). Compost happens either aerobically or anaerobically when organic materials are mixed and piled together. Aerobic composting is the most efficient form of decomposition and produces finished compost in the shortest time. Microbes break down organic compounds to obtain energy to carry on life processes. Under aerobic conditions, the "heat" generated in composting is a by-product of biologic burning, or aerobic oxidation of organic matter to carbon dioxide. If the proper amounts of food (carbon), water, and air are provided, aerobic organisms will dominate the compost pile and decompose the raw organic materials most efficiently. Optimal conditions for rapid, aerobic composting include carbon-nitrogen (C:N) ratio of combined feed stocks between 25:1 and 35:1, moisture content between 45% and 60% by weight, available oxygen concentration greater than 5%, feedstock particle size no greater than 1 inch, bulk density less than 1,000 pounds per cubic yard, and pH between 5.5 and 8.5 (Leslie, 2000).

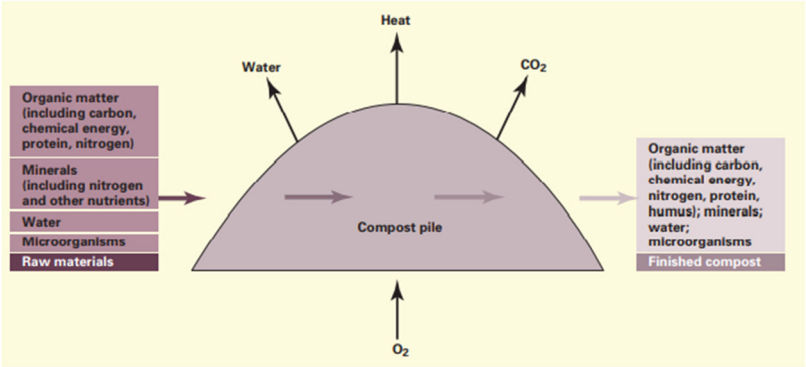


Figure 3.2. Schematic of Composting Process (Rynk, 1992)

The composting process can be divided into two main periods: (1) active composting and (2) curing. *Active composting* is the period of vigorous microbial activity during which readily degradable material is decomposed as well as some of the more decay-resistant material, such as cellulose. *Curing* follows active composting and is characterized by a lower level of microbial activity and the further decomposition of the products of the active composting stage. When curing has reached its final stage, the compost is said to be stabilized.

The compost pile passes through a wide range of temperatures over the course of the active composting period. As the temperature varies, conditions become unsuitable for some micro-organisms while at the same time become ideal for others. The active composting period has three temperature ranges. These ranges are defined by the types of microorganisms that dominate the pile during those temperatures (fig. 3.3) and are called psychrophilic, mesophilic, and thermophilic. Psychrophilic temperatures are generally defined as those below 50 degrees Fahrenheit, mesophilic between 50 and 105 degrees Fahrenheit, and thermophilic above 105 degrees Fahrenheit.

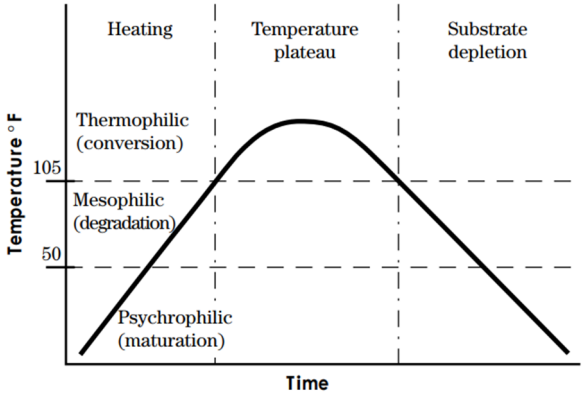


Figure 3.3. Compost Temperature Range

Composting is an aerobic process in general, but anaerobic microenvironments may develop. Atkinson et al. (1996b) estimated that almost 1% of all the bacteria found in municipal solid waste compost were anaerobic. All the anaerobic bacteria found were highly cellulolytic and thus may play a significant role in the degradation of macromolecules. The majority of the mesophilic anaerobic bacteria were facultative, while under thermophilic conditions more obligate anaerobic bacteria were found (Atkinson et al., 1996b).

3.2.2.1.Factors that Affect the Composting Process

Here are some of the factors that influence the composting process, including: substrates, environmental factors, and course of the process (Diaz et al., 2007):

a. Substrates

The complexity and nature of the molecular structure of the substrate are particularly important because these characteristics define the assimilability of the nutrients by the various

microorganism. The capacity of a microorganism to assimilate a particular substrate is a function of the microorganism ability to synthesize the enzymes responsible for breaking down complex compounds. The complex compounds are broken down into intermediate compounds or into an element that can be utilized by the microorganism in its metabolism and synthesis of new cellular material. In the event that all of the microbes do not have the necessary enzyme, the substrate basically remains in its original form.

The waste should contain all necessary nutrients. The macronutrients for microbes are carbon (C), Nitrogen (N), phosphorus (P), and potassium (K). Among micronutrients are cobalt (Co), magnesium (Mg), copper (Cu), and a number of other elements. Calcium (Ca) falls somewhere between the macro and the micronutrients. However, the principal role of Ca probably is as a buffer, to resist change in pH level.

One of the most important aspects of the total nutrient balance is the ratio of organic carbon to total nitrogen (C/N). A C/N in the starting material of about 25-30 is optimum for types of wastes. In a composting operation, the manifestation could require an excessively long time to reduce the C/N to a more suitable level. The optimum C/N is to some extent, a function of the nature of the wastes, especially of the carbonaceous components. If the carbon is bound in compounds broken down with difficulty by biological attack, its carbon accordingly would only become slowly available to the microbes. Compounds of this sort are chiefly lignin, some aromatics, and some physical forms of cellulose.

With respect to the nutritional needs of the microbe's active in composting, the C/N of the waste to be composted is the most important factor that requires attention. Experience shows that almost without exception, all other nutrients are present in typical organic waste in adequate amounts and ratios.

b. Environmental Factors

Composting is a biological process, it is fundamentally affected by the collection of environmental factors that determine the course of action in all biological systems. The principal environmental factors of interest in composting are temperature, pH, aeration, moisture, and substrate.

To have a high rate of biodegradation and a maximum microbial diversity, the temperature must range between 30-45 degrees Celsius. During the composting process, in order to minimize the retention time, a feedback temperature control can be operated with a set point between 30 and 45 degrees Celsius.

Another very important environmental factor is pH. Optimum range of pH organic matter that can be composted is 5.5-8. Whereas bacteria prefer a nearly neutral pH, fungi develop better in

a fairly acidic environment. In practise, the pH level in a composting mass cannot be change easily. Generally, the pH begins to drop at the beginning of the process (down to 5.0) as the consequence of the activity of acid forming bacteria that break down complex carbonaceous material to organic acids as intermediate product.

Apart from temperature and pH, other factors are moisture and aeration. Water is essential for all microbial activity and should be present in appropriate amounts throughout the composting cycle. Optimal moisture content in the starting material varies and essentially depends on physical state and size of particles and on the composting system used. Normally, a 60% moisture content in the starting material should be satisfactory. Because different materials have different water holding capacities, an exact generalization cannot be made optimal starting or time-course moisture levels.

Excessive water tends to plug pores and impedes gas exchange. However, a proper balance between the needs for available water and gas exchange should be maintained. Excessive moisture in the starting material could favour anaerobic processes, resulting in a slower process and low quality final product.

3.2.2.2.Composting Types

There are many types of composting and each method has different features. Generally, there are two major types of composting namely anaerobic and aerobic. The first method is processed in the absence of oxygen and the second one is processed in the presence of oxygen. Anaerobic composting is less labour intensive as it does not require frequent turning of the pile. It is a low-temperature process because the process of decomposition by anaerobic bacteria requires less energy released (Gaschk et al., 2009). However, unlike aerobic composting, anaerobic compost maintains both seeds of weeds and pathogenic microorganisms because the temperature produced is not high enough to kill them. Hence, it is more unsanitary and there might be higher incidence of serious diseases occurred. Moreover, anaerobic composting decomposes at a very slow rate and produces a strong odour.

Conversely, aerobic composting is a high-temperature process because the process of oxidizing compounds requires more energy. However, the temperature will decrease gradually after intense activity of microorganisms. Therefore, occasional monitoring of moisture and temperature are needed to maintain air circulation during this process. Therefore, it will need more labours to mix the pile thoroughly. However, the process of decomposing is faster than anaerobic methods and there is less unpleasant odour (Henry et al., 2005). People usually show their resistance towards home composting due to the particular smell or the presence of flies (Ali et al., 2004). Hence, aerobic composting may achieve higher public acceptance by local people. Composting method continues to attract more users not only due to being an environmentally sustainable product but also for its

great qualities as soil amendment. Compost utilization will assist to improve soil quality and reduces soil loss, and also increases soil water retention and reduce the need for extra inputs (USCC, 2008).

3.2.2.3.Composting Methods (Misra et al., 2003)

There is an extensive literature on composting methodology. A broad distinction as "Traditional" and 'Rapid' composting practices has been made, based mainly on the considerations of the practices being adopted as a convention; and the recent introductions for expediting the process, involving individual or combined application of treatments like shredding and frequent turning, mineral nitrogen compounds, effective microorganisms, use of worms, cellulolytic organisms, forced aeration, forced aeration and mechanical turnings and so on.

By and large, 'Traditional Methods' adopt an approach of anaerobic decomposition, or aerobic decomposition based on passive aeration through measures like little and infrequent turnings or static aeration provisions like perforated poles/pipes, and are time taking processes involving several months. On the other hand, 'Rapid Methods' make use of the treatments introduced recently such as those mentioned above to expedite the aerobic decomposition process and bring down the composting period around four to five weeks.

Besides, there are certain other recently introduced approaches like 'Vermi Composting', which though bring down the process duration to a good extent as compared to the conventional methods besides producing a far-superior quality product, have a lower turnover and longer time taken as compared to other 'Rapid Methods'.

Traditional methods based on passive composting approach involve simply stacking the material in piles or pits to decompose over a long period with little agitation and management. 'Indian Bangalore method' relying on this approach permits anaerobic decomposition for a larger part of operations and requires six to eight months for the operations to complete. The method is still in use in the urban areas of the developing world, mostly for treatment of urban wastes. A method similar in approach involving anaerobic decomposition and followed in western globe with large farms, is the 'Passive Composting of Manure Piles'. The active composting period in this process may range from one to two years.

'Indian Indore method', which slightly enhances passive aeration through a few turnings - thereby permitting aerobic decomposition; reduces the time requirement; and enables production in a time-span of around four months. Chinese rural composting methods, based on passive aeration approach through turnings/ aeration holes, provide output in two to three months. The methods are extensively used in developing world. Though the labour requirements for these methods are high, they are not capital intensive and do not require sophisticated infrastructure and machinery. Small

farmers find them easy to practice, especially in those situations where manual labour is not a constraint. However, the low turnover and longer time span are the major bottlenecks.

'Turned Windrows' have been in use with the large farms especially in the developed parts of the world. The windrows are periodically turned using a bucket loader or special turning machine, commonly available on these farms. The turning operation mixes the composting materials, enhances passive aeration and provides conditions congenial for aerobic decomposition. Composting operations may take up to eight weeks. 'Passively Aerated Windrows' eliminate the need for turning by providing air to the materials via pipes, which serve as air ducts. Active composting period could range between ten to twelve weeks.

Rapid methods like 'Berkley Rapid Composting' and 'North Dakota State University Hot Composting' involve accelerated aerobic decomposition through measures like chopping of raw materials to small size; use of mineral compounds like ammonium sulphate, chicken manure, urine; and turning of the material on daily basis. While chopping without much machinery support may be possible at smaller scales, mechanisation may be necessary at large scale applications. Whereas 'Berkley Rapid Composting' methods claims an active composting period of two to three weeks only, 'North Dakota State University Hot Composting' may take four to six weeks.

'EM based Quick Compost Process' involves aerobic decomposition of rice husk/bran and cow dung as raw materials in pits as enabled through turnings; and uses effective microorganisms as activator for expediting the decomposition process. The use of EM as activator brings down the composting period requirement from twelve weeks to four weeks.

An example of cellulolytic culture based method is the 'IBS Rapid Composting' which is a development of windrow type of composting. Salient process features include chopping of vegetative organic materials, passive aeration provisions through air ducts and use of cellulose decomposer fungus (*Trichoderma harzianum*).The process requires about four weeks.

Mechanical forced aeration based methods like 'Aerated Static Pile' reduce the composting time period further, allow for higher, broader piles and have lower land requirements as well in comparison to 'Windrow' or 'Passively Aerated Windrow' methods. However, there is little experience using 'Aerated Static Piles' with agricultural wastes. The technology is commonly used for treatment of municipal sewage sludges. Active composting period may range between three to five weeks.

Mechanical forced aeration and accelerated mechanical turning methods like 'In-vessel composting ' are specially designed commercial systems, with potential advantages like reduced labour, weather proofing, effective process control, faster composting, Reduced land requirement, and quality output. However, high investment and recurring costs related to operation and

maintenance could be a bottleneck for adoption especially in economically backward areas. Among the systems, 'Bins' and 'Rectangular Agitated Beds', in particular, have found place on several large farms in developed world. Bin composting involves provisions for forced aeration in the bin floor; little turning of the composting material; and movement of material from one bin to another. 'Agitated Bed Systems' appear to have promise for on-farm rapid composting. However, the cost for the system is expensive. Commercially manufactured large systems (150 tons/day or larger) reportedly available through; small systems (20 tons/day or less) likely to interest the majority of farmers, are lacking.

'Vermicomposting' based on the use of worms results in high quality compost. The process does not require physical turning of the material. To maintain aerobic conditions and limit the temperature rise, the bed or pile of materials needs to be of limited size. Temperatures should be regulated so as to favour growth and activity of worms. Composting period is longer as compared to other rapid methods and varies between six weeks to twelve weeks.

3.2.3. Soft-rot fungi

The majority of fungi are mesophiles which grow between 5°C and 37°C, with an optimum temperature of 25-30°C (Dix and Webster, 1995). However, in the compost environment the elevated temperature means that the small group of thermophilic fungi is an important biodegradation agent. In nature, thermophilic fungi grow in garden compost heaps, bird nests, coal tips, power plant cooling pipes and effluents, in the storage of many agricultural products (like hay, grain etc.) and in piles of wood chips and peat (Sharma, 1989; Dix and Webster, 1995). In mushroom compost, thermophilic fungi are responsible for the degradation of lignocellulose, which is a prerequisite for the growth of the edible fungus (Sharma, 1989).

Soft-rot fungi degrade wood in environments that are too severe for white- or brown-rot fungi, generally in wet environments (Blanchette, 1995). They also decompose plant litter in soils. Haider and Trojanowski (1980) and Rodriguez et al. (1996) studied the lignin mineralization capacity of soft-rot fungi. In 35 days, *Chaetomium piluliferum* mineralized 17% of ring14C-labelled corn stalk lignin, but the amount mineralized by other soft-rot fungi was much less (Haider and Trojanowski, 1980). Mineralization of side chain-and methoxy-labelled lignins by all fungi was slightly higher (Haider and Trojanowski, 1980). The soil fungi *Penicillium chrysogenum*, *Fusarium solani* and *Fusarium oxysporum* mineralized 20-27% of 14C-MWL from wheat straw in 28 days. P. chrysogenum can also attack kraft and organosolv lignins (Rodriguez et al., 1996). Little is known about the enzyme system of soft-rot fungi or their lignin degradation capacity as litter decomposing organisms (Haider and Trojanowski, 1980; Kirk and Farrell, 1987). Lignin-related compounds, like

vanillic acid and phenols, are rapidly degraded by softrot fungi (Haider and Trojanowski, 1980). This could mean that soft-rot fungi are an important group of lignin degraders in mixed populations, although their lignin degradation capacity alone is limited. The adaptation of soft-rot fungi in various temperature, different pH, and limited oxygen is higher than other fungi. Soft rot fungi are no doubt the most efficient fungi to degrade lignin in mixed microbial population.

Fungi have two types of degradation systems: intracellular, together with outer cell envelope layer, and extracellular, important for polysaccharide degradation. Furthermore, the extracellular enzymatic system includes two types of enzyme: hydrolytic, responsible for polysaccharide degradation; and oxidative, which degrade lignin and open phenyl rings. Degradation of soft rot fungi leads to darkening organic material by the produced laccases, and peroxidases involved in lignin modifications (Sanches et al., 2009). Degradation of lignin is more efficient than in the case of brown rot and soft rot fungi, because they possess a unique ability to its complete mineralization to CO_2 (Couturier et al., 2013).

3.2.3.1.Penicillium spp

Penicillium belongs to the phylum Ascomycota, however its taxonomic characterization is still a matter of discussion (Grim et al., 2005) and the difficulties in identifying most *Penicillium* species requires multidisciplinary approaches. Clarification of species concepts in the genus *Penicillium* was supported mainly by morphological characteristics. Raper and Thom, for example, based *Penicillium* taxonomy classification on the combination of macroscopical (such as colony texture and color) with micro morphological features (Raper et al., 1949). In spite of the fact this feature was regarded as subjective by Pitt and Stolk and Samson, the color of the conidial mass has been pointed out to be a species-specific characteristic that varies in concomitance with distinctive morphological features (Christensen et al., 1994; Pitt et al., 1979; Stolk et al., 1983). Dorge et al. (2000) proposed a method for direct identification of pure *Penicillium* species using image analysis. The various species of *Penicillium* can colonise many different environments. They are common in soils, in foods, in drinks and in indoor air (Banke et al., 1997).

The ability of most fungi to produce extracellular enzymes for the assimilation of complex carbohydrates without prior hydrolysis makes possible the degradation of a wide range of organic waste. They also have the advantage of being relatively easy to grow in fermenters, thus being suited for large scale production. Another advantage is the easy separation of fungal biomass by filtration due to its filamentous structure. In comparison to yeasts, filamentous fungi are less sensitive to variations in nutrients, aeration, pH, temperature and have a lower nucleic content in the biomass. In addition, several *Penicillium* strains have been shown to be able to live in saline environments, an advantage of these microorganisms over the others in the bioremediation field.

Although white root fungi (WRF) are well-known for their ligninolytic enzymatic activities and widely investigated for their utilization as advanced biological treatment options (Levin et al., 2004), one of the locally isolated filamentous fungi, *Penicillium sp*., appeared to be more efficient than the tested WRF, *T. chioneus, P. chrysosporium,* and *B. adusta* strains. This suggests that some of the natively grown filamentous fungal strains could have more biodegradation capability, and sometimes even better performance can be obtained than the WRF.

3.2.3.2.Aspergillus spp

The Aspergillus conidiophore consists of an elongated stalk or stipe culminating in an expanded bulbous region variously called the columella or vesicle on which are borne one or two layers of cells (variously called primary and secondary sterigmata, or phialides and metullae, respectively) that generate the asexual spores or conidia. The base of the conidiophore is 'T' or 'L' shaped and is called the foot cell, even though it is not a separate cell. The foot cell is a diagnostic feature of the *Aspergillus* conidiophore. Aspergillus is one of the most common molds on earth. There are over 200 species, all grouped together because they form similar asexual reproductive structures. Aspergillus species are capable of making vast numbers of conidiospores. These spores are clonal; thus one sporulating colony of *Aspergillus* makes millions of genetically identical propagules. In this fashion, *Aspergillus* 'sprinkles' the world with spores.

Most lignin research has been on wood-rot fungi and not on other lignolytic organisms. Members of the genus Aspergillus inhabit lignin-rich environments, and we have studied their relative lignin-degrading potential. *Aspergillus fumigatus, A. japonicus, A. niger,* and *A. terreus* were tested for their ability to metabolize 14C-labeled aromatic compounds. The species tested decarboxylated, demethoxylated, and cleaved the rings of coumaric, ferulic, vanillic, veratric, and anisic acids. More than 90% of 14C-ring-labeled ferulic and vanillic acids disappeared from the medium in 96 h of cultivation. More than half of the above was respired, the rest was incorporated in unknown form into the mycelium. Mycelia were homogenized and about 3% of the initial label was found in TCA precipitate of the cell-free supernatant. Protocatechuic acid 3,4-dioxygenase (EC 1.13.11.3) and catechol 1,2-dioxygenase (EC 1.13.11.1) activities were detected in the mycelial extracts of the *Aspergillus spp* (Milstein et al., 1984).

All the *Aspergillus spp.* were capable of degrading both aromatic and carbohydrate components of water-soluble lignocarbohydrate complexes (LCC) from wheat straw. The degradation of the aromatic moiety of soluble LCC with apparent molecular mass more than 100,000 daltons was far more active in the Aspergillus spp. than in the white-rot fungi tested: Polyporus versicolor, Pleurotus ostreatus, and Fomes annosus. The aromatics present in the soluble LCC, as

well as a variety of lignin-related compounds tested, did not affect the production of hemicellulases by *A. japonicas* (Milstein et al., 1984).

3.2.4. Fungal Delignification

A study of Kerem et al. (1992) compared lignocellulose degradation ability of two fungi: *Pleurotusostreatus and Phanerochaete. chrysosporium,* during solid state fermentation on cotton stalks as a substrate. The growth of *P. chrysosporium* resulted in the loss of 55% of the initial dry organic matter within 15 days of fermentation, while the lignin loss equalled to 35% of the initial lignin content in the substrate. The growth of *P. ostreatus* resulted in the loss of only 20% of original dry organic matter, while the lignin loss was 45% of the initial lignin content in the substrate. Li et al. (2001) analysed compositional changes of cottonseed hull substrate during *P. ostreatus* growth. After 45 days of incubation, lignin content decreased from an initial 17% to a final of 11% of dry matter. Moreover, they performed delignification of wheat straw by *Fusarium concolor* that is able to produce laccase, LiP and MnP enzymes when grow on a lignocellulosic medium. After 5 days of incubation they observed removal of 13.07% of the lignin and loss of 7.62% of the total polysaccharide fraction (Li et al., 2008).

Herpoel et al. (2002) investigated wheat straw pulp degradation combining commercial xylanases and laccases from *Pycnoporus cinnabarinus*, separately, followed by alkaline treatment. This two stage treatment was effective to remove 60% of lignin in wheat straw pulp. Also, xylanases and laccases, previously used for enzyme-aided bleaching, contribute improvements in the following chemical delignification step (Viikari, 1994). The same fungus was used in delignification of lignocellulose-containing raw materials (*Prosopis juliflora/Lantana camara*) reported by Gupta et al. (2011). The results showed that lignin removal improves the saccharification of cellulose. The fungal delignification was the highest during the first 15 days. An increase of 21.1–25.1% sugar release was obtained when fungal treated substrates were enzymatically hydrolysed as compared to the hydrolysis of untreated substrates.

3.2.5. Cow dung as a compost feedstock

Cows dung is a most important source of bio-fertilizer. Animals can play an important role in the provision of energy either in negative way where livestock keeping contributes to deforestation in large parts of forested area or in positively, such as by transforming plant energy into useful work or by providing dung used for fuel through dung cakes or biogas to replace charcoal, fuel wood, firewood etc. Most livestock products in mixed farming systems are derived from animals that are fed on local resources such as pasture, crop residues, fodder trees and shrubs. The

farm animals (cows, bullocks and milk buffaloes) provide dung and urine to enrich the soil, while crop residues and fodder form the bulk of the feed for these animals (Kesavan and Swaminathan, 2008).

There are a variety of cow dung and cow's urine products, which can be used as fertilizers and pest repellent respectively in agricultural practice. These products are very popular and are using day by day. Low soil fertility is one of the greatest biophysical constraints to production of agroforestry crops across the world (Ajayi, 2007). Cow dung is a very good source for maintaining the production capacity of soil and enhances the microbial population. But due to increasing population pressure and demand of food resources, there is a need of introducing a chemical fertilizer, pesticides and insecticides to the soil, which are disturbing the soil physiochemical properties including soil texture, porosity, and water holding capacity and also disturbed the soil microbial population. Therefore, improper use of cow dung should be stopped and should only be applied in the farmland instead of chemical fertilizers, so that the productivity and sustainability of soil could be maintained which will increase the production capacity of food treasure (Bargali, 2004).

Manure is an important input for maintaining and enhancing soil fertility. As per Fulhage (2000) manure contains the three major plant nutrients, nitrogen, phosphorus and potassium (NPK), as well as many essential nutrients such as Ca, Mg, S, Zn, B, Cu, Mn etc. That, in addition to supplying plant nutrients, manure generally improves soil tilth, aeration, and water holding capacity of the soil and promotes growth of beneficial soil organisms. The application of cow dung manure and vermicompost increases soil organic matter content, and this leads to improved water infiltration and water holding capacity as well as an increased cation exchange capacity. As per Mandal et al. (2013) integration of inorganic, organics and biofertilizers can produce 50-92% more yield in Aonla.

According to Adegunloye et al. (2007) C: N ratio in cow dung manure is an indication that it could be a good source of protein for the microbes which involved in decomposition of organic matter. Manure and urine raise the pH level and accelerate the decomposition of organic matter and termite activity (Brouwer and Powell, 1995, 1998). If inorganic fertilizer, especially nitrogen, is combined with manure, the manure reduces soil acidification and improves the nutrient buffering capacity and the release of nutrients (Williams et al., 1995). The soil productivity is also related to available nutrient source in either through manures (dung) or chemical fertilizers (superphosphate etc). Dung increased pH, CEC, total N, organic C, loss on ignition, and exchangeable Mg and Ca. It decreased sulphate sorption. Moreover, cow dung manure plays a significant role in maintaining the nutrient status of the plant. Vermicomposting of cow manure using earthworm species E. andrei (Atiyeh et al., 2000b) and E. foetida (Hand et al., 1988) favoured nitrification, resulting in the rapid conversion of ammonium-nitrogen to nitrate-nitrogen. Therefore it improves the nutrient

cycling and helping to convert unavailable nitrogen in available forms to plants. The soil biological attributes are also responsible for determination & maintenance of physical properties of soil.

The physical properties of soil in its own turn control not only the quantum of chemical properties, but also the rate of their release and availability to plants essential for metabolic processes. Thus, it may be said that soil biology is the door to maintenance of soil health (Kumari et al. 2014). As per Dinesh et al., (2000) there is a positive relationships between relevant soil properties and enzyme activities and suggested that addition of organic matter increased microbial activity/ diversity and turnover, which subsequently leads to greater enzyme synthesis and accumulation in the soil matrix.

3.2.6. Chicken manure as a compost feedstock

Chicken manure is preferred amongst other animal wastes because of its high concentration of macro-nutrients (Warman, 1986; Duncan, 2005). For example, Chescheir et al. (1986) found increase in nitrogen levels from 40 - 60% and 17 - 38% with respect to control for Norfolk sandy soils and Cecil sandy loam soils, respectively following application of manure. In addition, application of chicken manure to soil enhances concentration of water soluble salts in soil. Plants absorb plant nutrients in the form of soluble salts, but excessive accumulation of soluble salts (or soil salinity) suppresses plant growth. Stephenson et al. (1990) reported the EC of chicken manure of about 11 dS/m in silt loam soils too high for salinity sensitive crops.

The pH of dry chicken manure pellets was found to be 7.9, with most of the nutrients available in this environment (López-Masquera et al., 2008) while a decrease in the soil pH (< 6.5) affects the availability of nutrients to plants (Warman, 1986). For example, loses of grassland soils 23 - 35% of their total available bases (Ca, Mg, K, and Na) in England through acidification by animal manure were observed (Horswill et al., 2007). Wong et al. (1983) found that the acidity due to chicken manure addition severely affects root growth and seed germination. Moreover, if applied correctly chicken manure acts as a good soil amendment and/or fertilizer (e.g. provides N, P and K) and can also increase the soil and leaf N, P, K Ca, and Mg concentrations (Duncan, 2005; Agbede et al., 2008). These soil chemical properties provide information on the chemical reactions, processes controlling availability of nutrients and ways of replenishing them in soils (Prasad and Power, 1997).

3.3.Material and Method

3.3.1. Preparation of Fungi Starter

Fungi was isolated from fertile soil on Prefectural University of Hiroshima Japan, and then inoculated for 2 weeks in PDA (Potato Dextrose Agar) medium. A loopful of individual culture potential was taking up and inoculated in 100 g dried malt extract then added 1000 ml distilled water, autoclaved 121°C, and 20 minutes. Then cooling around 10 minutes. Stirring using Mixer MG-600 100 rpm, 36 hours. Then incubated 4 weeks, 25°C. That mix solution (Fig. 3.4) was used as the inoculum or activator (modification Gaind et al. 2009).

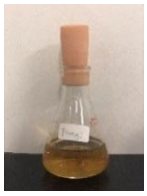


Figure 3.4. Fungi Starter

3.3.2. Preparation of Commercial Starter

Commercial activator that we used is pure activator, patented by the Bio Food Industry Research Center, and the Industrial technology center, Fukuoka Prefectural. Produced by the non-profit organization Eco cycle Kyushu / Okinawa Japan.

Figure 3.5. Commercial Starter

3.3.3. Preparation of Cow dung and Chicken Manure

Cow dung and chicken manure were used are commercial manufactured by Green Plant Factory Japan.

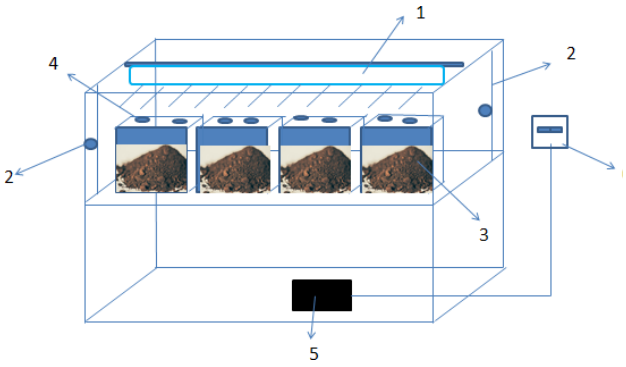
3.3.4. Treatments and Composting Procedure

This study was conducted in Prefectural University of Hiroshima, Shobara campus, Japan. Each sample was produced in 1-liter plastic container with small holes for air circulation. 3 samples (control, C1, and C2) then put in incubator with temperature 30 degrees Celsius. Description of sample as explains below:

1. Control: SCG 150 g, chicken manure and cow dung each 100 g and adding water until Moisture content around 60%.
2. C1: SCG 150 g, chicken manure and cow dung each 100 g, adding commercial (dilution 100 times with water) to sample until Moisture Content around 60%.
3. C2: SCG 150 g, chicken manure and cow dung each 100 g, then adding fungi activator (dilution 100 times with water) to sample until Moisture Content around 60%.

The amount of activator used is 1 percent of the total weight of water. Spent coffee grounds were fermented Robusta coffee from Jember, East Java, Indonesia. Then dried in oven laboratory at 60 degrees Celsius to remove all the humidity and inhibit microbial processes. After, these materials were mixed and used in composting to achieve a mixture with C/N ratio below 10 (for horticulture plant), the idea being obtain compost rich in nitrogen for plant fertilization.

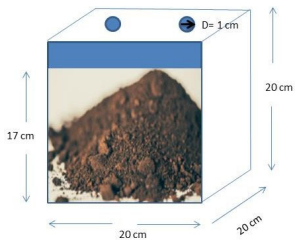
About 350 g dry material of each mixture was composted 28 days. The moisture was maintained around 60% of water content. The mixture was homogenized manually revolving each mixture almost every day. The temperature was controlled in 30 degree Celsius. Samples were taken on days 0, 7, 14, and 28 days. In all analyzes, 3 repetitions were performed for each sample. Sketch of the composting equipment can be seen in Figure 3.6 below.



a.

Information:

1. Lamp
2. Ventilator
3. Materials
4. Small hole
5. Temperature control
6. Electric socket



b.

Information:

Length 20 cm, width 20 cm, height 20 cm, stack height of material 17 cm, diameter of each whole 1 cm

Figure 3.6. (a) Composting equipment with temperature control, (b) box of compost

3.3.5. Physical and Chemical Analysis

3.3.5.1.Physical and Chemical Analysis of Raw Material

Before composting is carried out screening of raw materials first, including analysis of cellulose, lignin, caffeine, and protein. Analysis of cellulose, and lignin, which is 200 mg of raw materials (Spent Coffee Grounds, cow dung, and chicken manure), added 1 M H_2SO_4 1 ml mixed and put to water bath shaker 1 h. Then it was autoclaved 1 hours 121 degree Celsius. Then it was filtrated using filter paper. Weighed sample as hemicellulose. Then put in oven 60 degrees Celsius. Weight the solids as lignin. Total of Polyphenol analysis uses spectrophotometry method (AOAC, 2002). Analysis of protein use Lowry method (AOAC, 2002). In addition, screening of macro and micronutrient content related to their potential to be composted was also carried out.

3.3.5.2.Physical and Chemical Analysis during Composting

The following analysis was carried out in fresh compost samples: electrical conductivity (EC), and pH were determined by "Soil analysis" standard procedures (Cen, 1999); Total carbon and total nitrogen were determined also in dry sample by Macro corder (JM 1000CN). Then, the C/N ratio was calculated.

3.3.5.3.Macro Nutrient Analysis

The concentration of element macro nutrient Ca, Mg, P, and K were measured in dry samples by ICP-OES (inductively coupled plasma optical emission spectrometry) (Hitachi, PS7800) after nitric per chloric acid digestion (Cen, 1999).

3.3.5.4.Amount of Microorganism

Amount of microorganism was measured using Petri film (3M made in USA). Compost sample (30 g) while activator samples (30 ml) were diluted in 270 ml distilled water that has been autoclaved. Then diluted with took 10 ml to other conical, added distilled water 90 ml. The evaluation concentration of microorganism was determined by serials dilutions according to equation: (Rebollido et al. 2008)

CFU/g= Colonies number x dilution x 100

3.3.5.5.Morphology of Activator

Morphology of fungi was observed under microscope using slide culture. While commercial activator was observed using Scanning Electron Microscopy (SEM) JSM-7900F Schottky field emission (Kalab et al., 2008).

3.3.5.6.Relative Enzyme Activity of Fungi

Each Fungi isolates obtained were grown on the minimal agar medium supplemented with 0.5% CMC (carboxyl methyl cellulose), 0.5% caffeine, and 0.5% lignin (pH 7.0) at 30^{o} C for 4-5

days. The plates were then flooded with 0.33% iod solution followed. The appearance of a zone of hydrolysis around the colonies indicates synthesis of extracellular celluloses, xylanase by the microbes and fungus. Enzyme activity potential of the positive isolates was evaluated by measuring Relative Enzyme Activity (REA), i.e., ratio of diameter of zone of hydrolysis to the diameter of the colony (Sreedevi et al., 2013).

3.3.5.7.Germination Index

Percentage of seed germination, root growth and germination index (GI, a factor determined by both germination & root growth) were calculated based on the formula (Zucconi et al. 1981):

Seed germination (SG %) = SG % in each extracts/SG % in control x 100

Root growth (RG %) = mean root length in each extracts/mean root length in control x 100.

Germination index (GI) = multiplying SG % and RG %

3.3.5.8.Functional Group identification (by FTIR)

Functional group analysis is carried out by means of Thermo Scientific Nicolet iS20 FTIR-ATR Japan (Attenuated Total Reflection-Fourier Transform Infra-Red) (Meissl, 2008).

3.4.Result and Discussion

3.4.1. Chemical Composition in Raw Materials

Spent coffee ground (SCG) contains many polysaccharides such as hemicellulose (39.75%), and lignin (23.1%). Cow dung and chicken manure also contain hemicellulose and lignin which are also high. Cow dung has 18% hemicellulose, and 8.9% lignin, while chicken manure has 11% hemicellulose and 13.75% lignin (Table 3.1). These values are comparable to the others reported in the literature for SCG (Musatto et al. 2011a, 2011b). A little difference with some of the results of other studies may differ the type of coffee, method of roasting and also brewing techniques. It is very suitable for composting, mixing with spent coffee grounds to prevent nitrogen loss. The composition of manure is highly variable, according to animal type, diet, type of housing, and the amount and type of litter, and spilling water used.

The high content of hemicellulose and lignin is the basis for choosing the type of fungi stater in composting, namely *Aspergillus spp* and *Penicillium spp*. Lignin is a major structural component of plants and is the one that is degraded the slowest. It has been claimed that humus is mainly formed from lignin, polysaccharides and nitrogenous compounds (Varadachari and Ghosh, 1984; Fustec et al., 1989; Inbar et al., 1989). Therefore, in this study we choose specific fungus that can degraded lignin very well. The degradation of lignin is primarily accomplished by fungi. For hemicellulose, the main degrading enzyme is xylanase, produced by many bacteria

and fungi (Diaz, 2007).

This study also analyzed the caffeine content and total polyphenols in SCG. This is because both of these compounds are toxic to soil and plants (Cruz et al., 2014). The caffeine content in the SCG sample was 1.83%. This value is lower than previous research of Musatto et al (2011), because the sample used is Robusta coffee that has been fermented. This fermentation process causes caffeine to degrade and its value is lower than other references.

Hakil et al (1998) mention that *Penicillium* and *Aspergillus* are the more frequent caffeine-degrading genuses. It therefore seems logical that the majority of the studies done on caffeine degradation by filamentous fungi are related to Aspergillus and Penicillium genuses.

Table 3.1. Chemical Content in Raw Materials

Chemical content	SCG (%)	Cow dung (%)	Chicken manure (%)
1. Hemicellulose	39.75	15.6	26.7
2. Lignin	23.1	9.05	10.2
3. Caffeine	1.83	n/a	n/a
4. Protein	10.82	n/a	n/a
5. Total phenolic	1.61	n/a	n/a

Data are expressed as mean of three replicates. n/a – not analyzed

SCG also contains Carbon and Nitrogen ratios 19.5/1. Cow dung (12.36/1), and chicken manure (9/1) (Table 3.2) which approaches the C / N ratio of the soil, 20/1. Thus, SCG, cow dung, and chicken manure have the potential to be used as compost products because it has the macro and micronutrients needed by soil and plants.

Table 3.2. Macro nutrient content of Raw Materials

Macro nutrient content	SCG (%)	Cow dung (%)	Chicken manure (%)
1. Carbon	46.24	29.56	19.36
2. Nitrogen	2.37	2.39	2.15
3. C/N ratio	19.51/1	12.36/1	9/1

Data are expressed as mean of three replicates

3.4.2. Selection Microorganism for Composting

2 fungal isolates from fertile soil were screened for their ability to produce celluloses. We also observed morphology of each strains. Fungi were observed under a light microscope with a magnification of 40x (Fig. 3.7 and 3.8). This is because fungi have large cell sizes. From these observations, it is known that fungi are *Aspergillus sp* following characteristics: colony grows quickly, green color of colony, single row of phialides covering entire vesicle, conidiophore point out in all direction, and in variable length Rough, pitted, spiny. Besides, in activator also consist of *Penicillium sp*, with characteristic: old green color. Conidiophore hyaline upright, branched, tapered phialide, conidia pale green-shape ellipse or sub globose and single celled. This is in accordance with morphology *Penicillium sp.* which was described by Watanabe (2002).

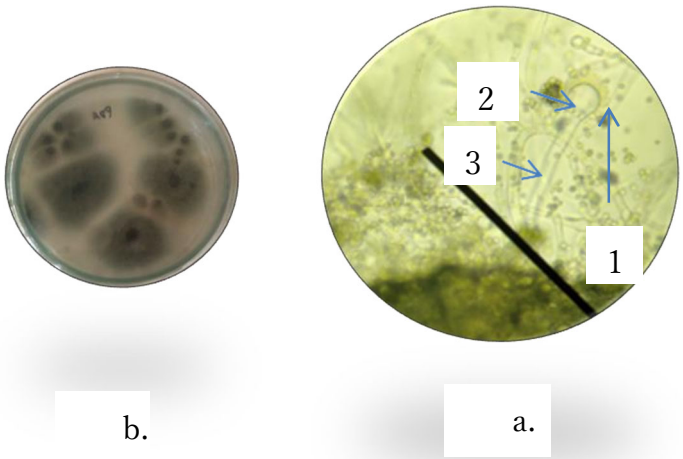


Figure 3.7. (a). Macroscopic and microscopic *Aspergillus sp,* green color of colony, (b). Morphology of Aspergillus with magnification 40x (1) conidiospore, (2) vesicule, (3) conidiophore

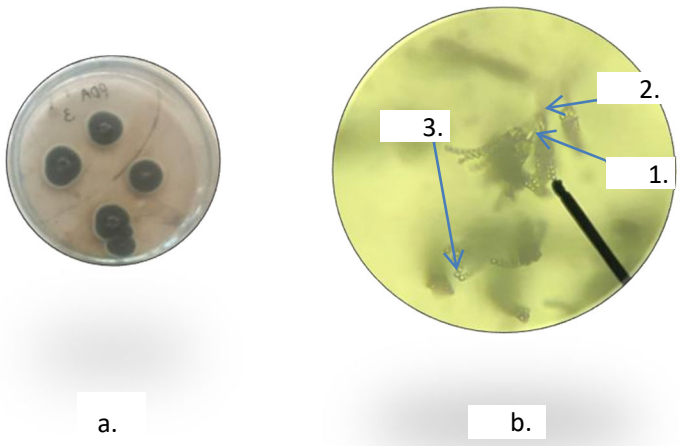


Figure 3.8. (a). Macroscopic and microscopic *Penicillium sp*, old green colour of colony, (b). Morphology of *Penicillium sp* with magnification 40x (1) Phialide, (2) conidiophore, (3) conidium

For quantitative screening, Results present showed positive cellulose producers for plates flooded with Iodine solution. Clearing zones surrounding microbial growing colonies after incubating for a suitable period indicating their ability for cellulose production. Kasana et al. (2008) discovered that Gram's Iodine for plate flooding in place of hexadecyl trimethyl ammonium bromide or Congo red, gave a more rapid and highly apparent result. Gram iodine was also used for the screening of cellulose producing microorganisms, i.e., fungi (Shahriarinour et al., 2011) and bacteria (Sreedevi et al., 2013). The Relative Enzyme Activity (REA) was measured by comparing the diameter of the clear zone and the diameter of the colony. The REA measurement results are presented in Table 3.3 below:

Table 3.3. Relative Enzyme Activity (REA) of fungi

Sample	REA to degrade CMC (cm/cm)	REA to degrade caffeine (cm/cm)	REA to degrade Xylan (cm/cm)	REA to degrade lignin (cm/cm)
Aspergillus sp	7.82	5.17	2.17	10.5
Penicillium sp	15.57	13	2.4	18.35

Data are expressed as mean of three replicates

From the table above, *Penicillium sp* has a greater REA than *Aspergillus sp.* combining these 2 isolates is expected to increase the production of cellulase enzymes so as to accelerate macromolecular degradation. According to Subowo & Corazon (2010) and Subowo (2015) that *Aspergillus sp.* and *Penicillium sp.* has the ability to break down lignin, as well.

3.4.3. Chemical Changes during Composting

In the composting process, to determine the maturity, stability and quality of compost is to observe chemical changes in the material. Chemical changes in the material during composting process are described below. Observation of each parameter is done for the initial sample, 7, 14, 21, and 28 days composting is carried out aerobically and the sample is left in a container at incubator 30 degree Celsius.

3.4.3.1. pH and EC

pH is monitored continuously every week because pH fluctuations indicate a degradation process organic compounds by microorganisms. Results of compost pH can be seen in Figure 3.9. pH conditions during the composting process change. On day 0 of composting, the pH was slightly acidic (6.72-6.75). On the 7th day, then the pH increased to reach pH 8 and more. Then the pH decreased to reach almost pH 7 or slightly basidic until in the end of the composting process. This is in accordance with the opinion of Marlina (2009) and Haga (1990) who stated that at the

beginning of composting the pH of the compost material was acidic. With the continuation of the composting process, the number of microorganisms increases and produces organic acids thereby lowering the pH. Furthermore, microorganisms begin to convert inorganic nitrogen into ammonium which causes pH to increase rapidly and compost becomes basidic, in the first and second weeks. Kim et al., 2007 in Caceres et al., 2018 also stated that at the beginning of composting the pH rises Ammonia as a result of nitrogen decomposition which is also affected by temperature. Some of the ammonia is released or converted to nitrate, then nitrate is denitrified by bacteria so that the compost pH becomes neutral at the end of composting. This indicates mature compost suitable for most cultivated crops (Lasaridi and Stentiford, 1998).

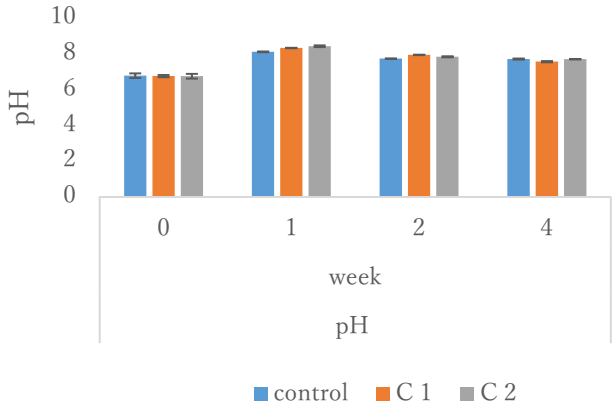


Figure 3.9. pH changes during composting

Electrical conductivity (EC) is associated with the release of easily decomposable compounds into the solution and indicates if the account for the total soluble ions in composts may endanger the quality of compost used as fertilizer. The EC values of matured compost samples in the range of 2.19-9.32 ms/cm. The evolution of EC for the three samples of composts is presented in Figure 7. The general tendency of EC for all three samples of composts was to increase during the composting process. Usually a higher value of EC could be an indication of high nutrient elements presence, or a slower decomposition of the organic matter therefore a lower release of mineral salts into the solution in the process of biodegradation of biomass waste (Barberis et al., 1996; Butler et al., 2001).

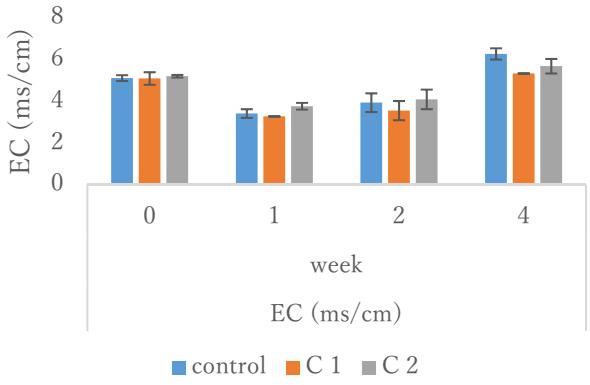


Figure 3.10. EC changes during composting

3.4.3.2. C/N ratio

The total of C content of the compost SCG was decrease during composting time. Because of the reduction in available carbon sources and synthesis reactions of the new complex and polymerized organic compounds or humification during the maturation phase (Bernal et al. 2009). Some researchers also indicated that the organic carbon content of compost samples has decreased during composting (Kassegn et al. 2015; Getahun et al. 2012b; Dadi et al. 2019). As shown in Fig. 11, it can be seen that after one month the carbon has decreased. Compost sample with C2 has lowest total carbon average in final composting (6,99%), followed by C1 (7,04%) and control (8,33%). This means, C2 with fungi activator has the ability to degrade carbon better than others. Decreasing carbon of Sample C1 as much as 61.81%, while C2 60,56%. By using these three types activator, carbon reduction is better when compared to controls 56.51%. Our activator can compete with commercial. It is because the difference in the percentage of carbon reduction is only about 1.25%.

Decomposition rate of each compost can be seen in Figure 3.11. In the first week (mesophilic phase), C2 activator has a higher decomposition rate. Optimum temperature for *Aspergillus* and *Penicillium spp* growth is 28-37 degrees Celsius (Singh et al., 2014). This indicates composting with a temperature of 30 degrees Celsius, very good for the material using a fungi activator. This species are also able to grow in a wide range of pH from 3.0 to 7.0 with maximal growth rates at 4.0, the optimum range being 4.6 to 6.8 (Singh et al., 2014). At the end of composting, all three samples had almost the same decomposition rate of carbon.

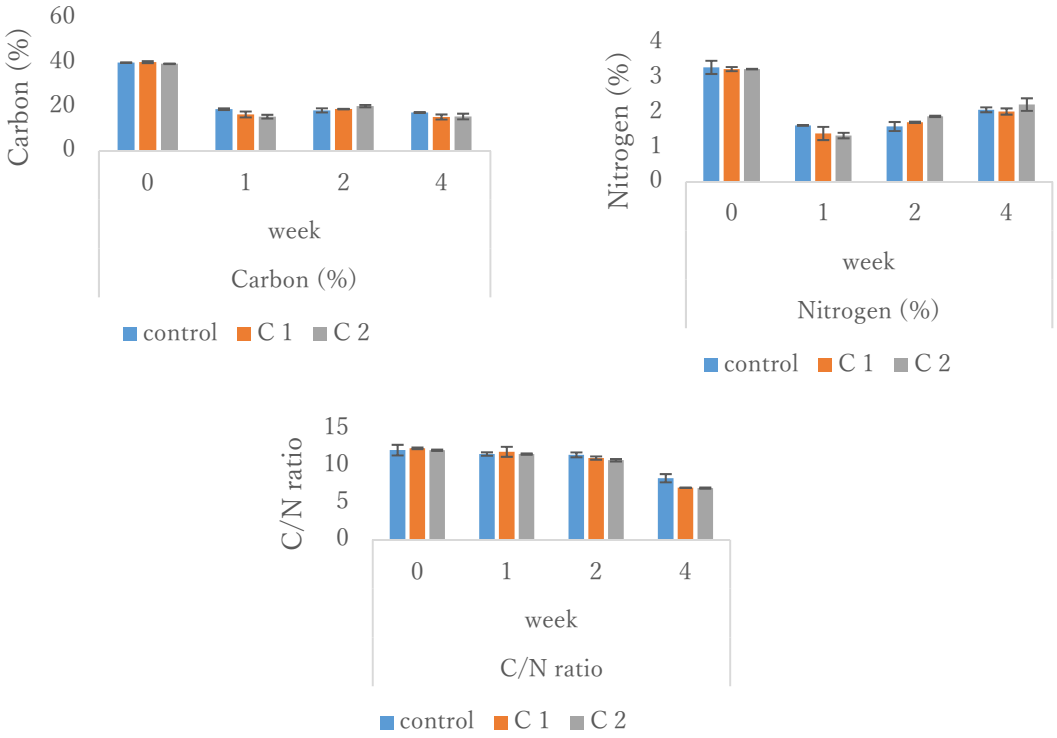


Figure 3.11. C/N ratio during Composting

Total Nitrogen (TN) includes both organic nitrogen and inorganic nitrogen (mainly ammonia nitrogen and nitrate) which are normally assimilated by microbes. The variations of TN for different treatments are also shown in Fig. 8. In the first week when the pH is alkaline, so there is an ammonification process in which inorganic N turns into ammonium. In Figure 11 above, nitrogen has dropped dramatically. However, in the second and fourth weeks, total nitrogen showed a slight increase. This is because the pH begins to return to near normal, there is nitrification where the ammonium changes to nitrate. Total N (TN) increased slightly as a consequence of concentration effect due to the mass loss during composting (Bernal et al. 2009). As NH_4^+-N could be easily transformed to organic forms under the metabolism of microorganisms or lost through NH_3 volatilization, there was decrease of NH_4^+-N concentration at the final of composting, which could be explained by volatilization and immobilization processes. As we can see in Figure 8, it shows the total nitrogen which has decreased compared to the initial.

3.4.3.2. NH_4^+-N/NO_3^- N ratio

Ammonium nitrate ratio is also one of parameter to check maturity of compost. The decrease of NH_4^+-N and appearance of NO_3^- -N are good indicators of the maturation process (Riffaldi et al. 1986).

NH_4^+-N / NO_3^- -N ratio decreased gradually during composting and reached near 0,1 in the final compost (Fig. 3.12), which was almost same with previous study, the value (<0.16) established

by Bernal et al. (1998) for mature compost, but lower than the result found by Huang et al. (2004). As different composting technologies and raw materials were used, the value varied at a wide range (Bernal et al. 1998; Huang et al. 2004; Amir et al. 2005; Haroun et al. 2009).

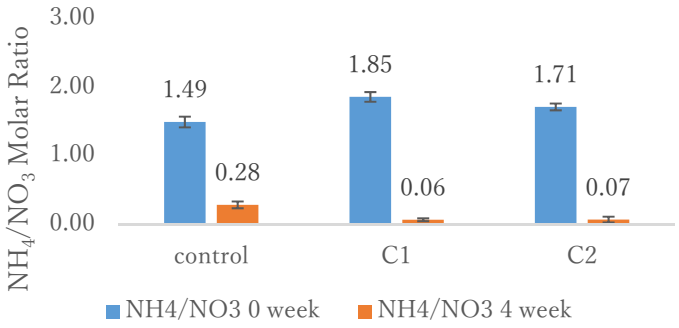


Figure 3.12. Ammonium-Nitrate ratio after composting

3.4.4. Degradation macromolecule content of Material

On the world lignocelluloses are the main part of biomass, because it is a renewable resource and the prominent structural component of plant cell wall as well. Cellulose is the dominant part of lignocellulose and consists of a linear chain of D-glucose linked by β (1-4)- glycosidic bonds to each other. The cellulose strains are connected to each other deliver cellulose fibril. A number of intra- and intermolecular hydrogen bonds are linked cellulose fibers together. Hemicellulose is the second plentiful constituent of lignocellulose, is comprised of diverse pentoses (arabinose, xylose) and hexoses (mannose, galactose, glucose) (Alvira et al., 2011).

Previous study have explained about transformation of macromolecule such as cellulose, hemicellulose, and lignin as a consequence of biological activity during composting (Caricasole et al. 2011, Zhao et al. 2011a,b, Bikovens et al. 2012, Hachicha et al. 2012, Luz Cayuela et al. 2012, Wang et al. 2012a, Bernabé et al. 2013, He et al. 2013, Iwai et al. 2013, Paradelo et al. 2013). It has been claimed that humus is mainly formed from lignin, polysaccharides and nitrogenous compounds (Varadachari and Ghosh, 1984; Fustec et al., 1989; Inbar et al., 1989). Anothers studies also confirm the extensive loss of cellulose and hemicellulose, while confirming the increasing proportion of humus. Therefore, in this study we observed changes of cellulose, hemicellulose and lignin during composting.

Figure 3.13 summarized the rates of lignin, hemicellulose and cellulose decomposition during composting, and showed a relatively high initial proportion of cellulose (around 35.49- 69%) and a low lignin content (5.5 to 32.75%). The greater cellulose degradation in C2 could be explained by added to fungi when inoculating at t_0. This led to better global cellulose degradation in C2

(74.39%) compared to similar rates in C1 (83.47%). For lignin degradation, Sample C1 decreased 35.56%, Sample C2 40.28%. According to previous study, *Aspergillus* and *Penicillium* is a soft rot fungi which show preference for cellulose and hemicellulose. Soft rot fungi, can tolerate a wide range of temperature, humidity and pH conditions, and attack a variety of wood substrates (Blanchette et al., 2004). They are usually thought to degrade mainly carbohydrates in soil, forest litter and compost, but they may also degrade lignin in these environments (Rodriguez et al. 1996; Regalado et al. 1997; Tuomela et al. 2000; Kluczek-Turpeinen et al. 2003). Thus, some of them were found to be able to mineralize grass lignins (Haider and Trojanowski 1975) and e.g. *Penicillium chrysogenum, Fusarium oxysporum* and *F. solani* (Rodriguez et al. 1996) mineralized in 28 days up to 27% of a 14C- labelled lignin prepared from milled wheat straw. Unlike model white-rot basidiomycetes such as *Phanerochaete chrysosporium* and *Phlebia spp.,* which degrade lignin during secondary metabolism (Kirk and Farrell 1987; Hatakka 2001), the degradation by molds was maximal during primary metabolism (Regalado et al. 1997).

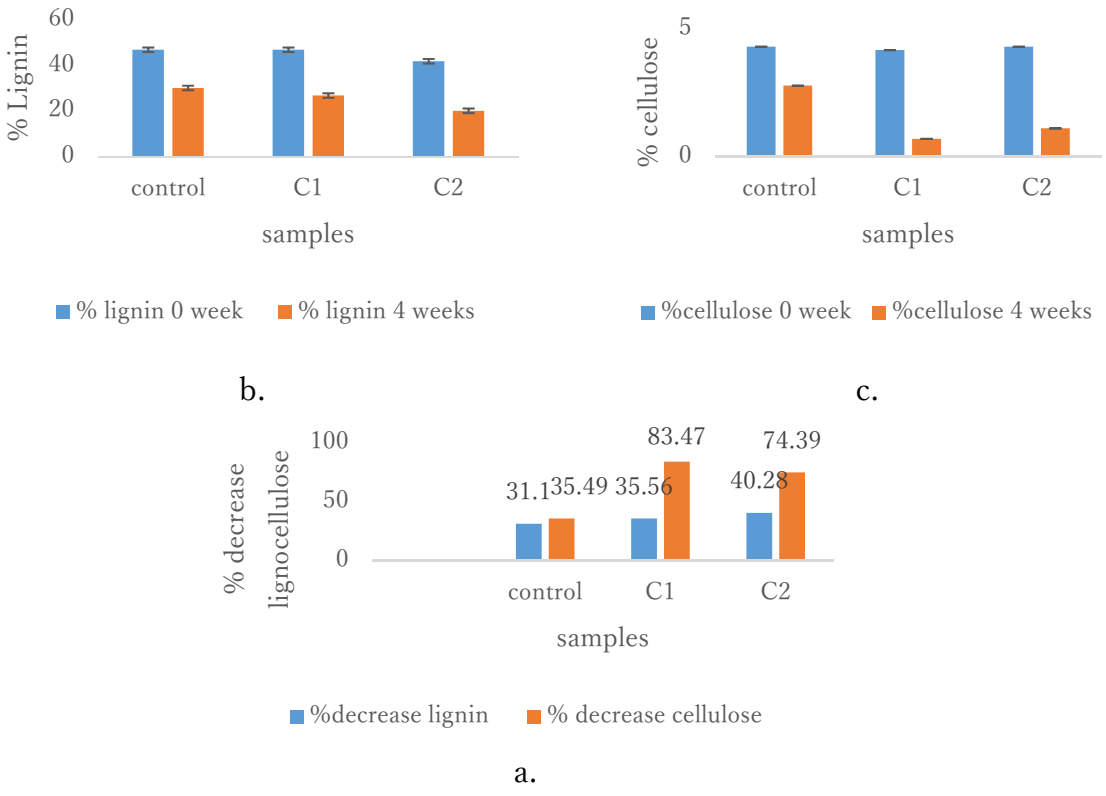


Figure 3.13. Decompose of lignocellulose after Composting

Protein is the first degraded to peptides by the enzymes proteases and then into amino acids by the enzymes peptidases. These enzymes are produced by many species of Bacillus. Further decomposition yields NH_3, NO_3, CO_2, and water. This process (ammonification) occurs as a result of hydrolitic and oxidative enzymatic reaction under aerobic conditions by heterotrophic microbes such as fungi and bacteria. As shown in Figure 3.14, the percentage of decompose of protein samples C2 and C1 is higher than the control of 85.44% and 83%.

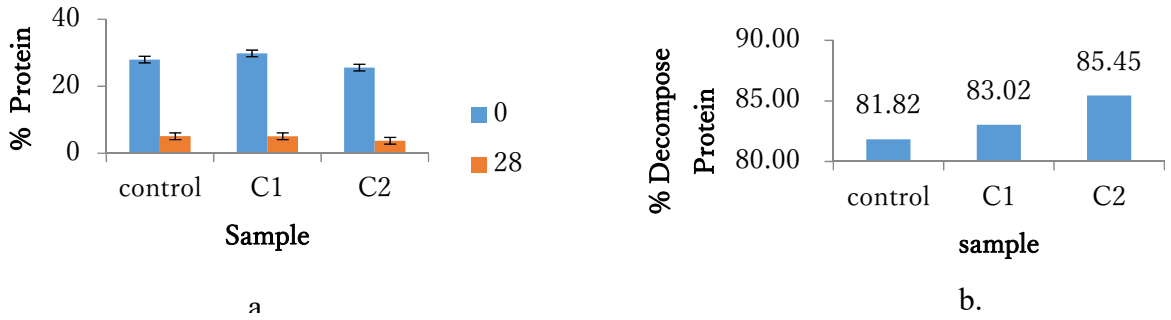


Figure 3.14. (a)% Protein, (b). % Decompose Protein during Composting.

3.4.5. Population of Microorganism

The degradation process of organic compound material is strongly influenced by the work of microorganisms during composting. Therefore the population of microorganisms during the composting process is also analysed. The results of the population of microorganisms are represented in Figure 15. At the initial or before composting, the material with the starter fungus (C2) had a higher number of microorganism populations (log 6.48 cfu /g), followed by C1 log 6.42 cfu /g, and control (C0) log 6.32 cfu /g. In the first week of composting, C1 has a higher population growth when compared to other samples, namely log 7.21 cfu/g. at the second week, sample C2 grew faster at log 7.49 cfu/g. Until the end of composting, sample C2 still has a high population of microorganisms, log 6.98 cfu/g.

Figure 12 shows the higher number of microbes, the decrease or decomposed C / N ratio is also sharper. Fungi have high cellulolytic and hemi cellulolytic activity, so they can decompose macromolecules and at the same time reduce the C / N ratio of the material. Bacteria also produce xylanase enzymes that can degrade hemicellulose (Diaz, 2007). In Figure 3.15, it also shows that as microbial numbers increase, cellulose decreases sharply in week 4. In this case, commercial activator (Bacillus) and fungi (Aspergillus have excellent ability in composting compared to control

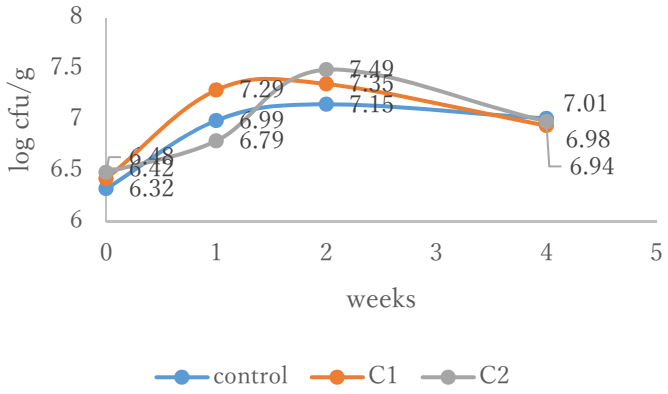


Figure 3.15. Population of Microorganism during Composting

3.4.6. Mineral

Phosphorus plays a role in cell division, fruit, flower and seed formation, plant maturity, stimulating the development of root hair, quality of crop yields and disease resistance (Goh and Hardter, 2003; Borooman and Grouh, 2012). Potassium plays a role in the physiological processes of plants such as enzyme activators, cell turgor regulation, nutrient transport and water and increase plant endurance (Goh and Hardter, 2003). Calcium is essential for the growth of meristems, and particularly for the proper growth and functioning of root tips. While magnesium is a specific constituent of chlorophyll, in which one atom of magnesium is bound to four pyrrole rings. Magnesium also plays a major role in numerous enzyme reactions (Alan, 1988).

Compost spent coffee ground has a high mineral element (Table 3.4.) and is very useful for plant growth. This value is higher than before composting. This increase in concentration is due to a decrease in carbon during composting, as in previous studies in the literature (Kulcu and Yaldiz, 2014; Liu and Price, 2011). This is an advantageof spent coffee ground compost with high mineral elements.

Table 3.4. Mineral element material and SCG compost

Sample	P (mg/g)	K (mg/g)	Ca (mg/g)	Mg (mg/g)
Initial material				
Control	2.71	3.72	9.59	2.93
C 1	3.28	3.72	9.71	2.53
C 2	3.25	3.69	9.95	5.22
Compost				
Control	2.42	3.73	12.36	7.83
C 1	5.64	3.75	21.18	9.54
C 2	3.29	4.15	40.28	8.07

Data are expressed as mean of three replicates

3.4.7. Phytotoxicity

The seed germination index (GI) has been defined as a factor of relative seed germination and relative root elongation (Moharana and Biswas 2016). It was reported that immature and unstable composts cause phytotoxicity afecting seed germination and root growth and thus result in lower seed GIs (Raj and Antil 2011; Awasthi et al. 2014). Previous research eforts show that a GI value of 80% indicates maturity of the compost and the absence of phytotoxicity (Tiquia 2005; Huang et al. 2006). All of samples showed non- phytotoxic (>80%) on germination test using Radish seeds (Table 5).

The results obtained indicated that all composts have matured within one month. By comparison, it was far faster than the normal duration of traditional composting (6 months to 1 year), vermicomposting (3-6 months) and Takakura home composting method (3 months). From Table 3.5, Sample C2 with Germination Index 191.86% is the best compare with others. While C1 have germination index 183.88%. This indicates from this method; all compost can produce healthier compost compare with compost control.

Table 3.5. RSG, RRE, and GI of compost

Sample	Seed Germination (SG) (%)	Root Elongation (RE)	Germination Index (GI) (%)
Control	90	102.66	92.39
C1	95	193.56	183.88
C2	100	191.86	191.86

Data are expressed as mean of three replicates

3.4.8. FTIR analysis

Infrared spectroscopy is based on interactions of infrared light with molecules or molecule groups. An infrared spectrum reflects the chemical composition of the sample like a chemical “fingerprint”. Theoretical assignments of absorption bands to functional groups have been summarized by several authors (Smith, 1999; Socrates, 2001; Bosh et al., 2002; Madejova, 2003). FTIR analysis results of the material after 1 month of composting can be seen in the Figure 3.16.

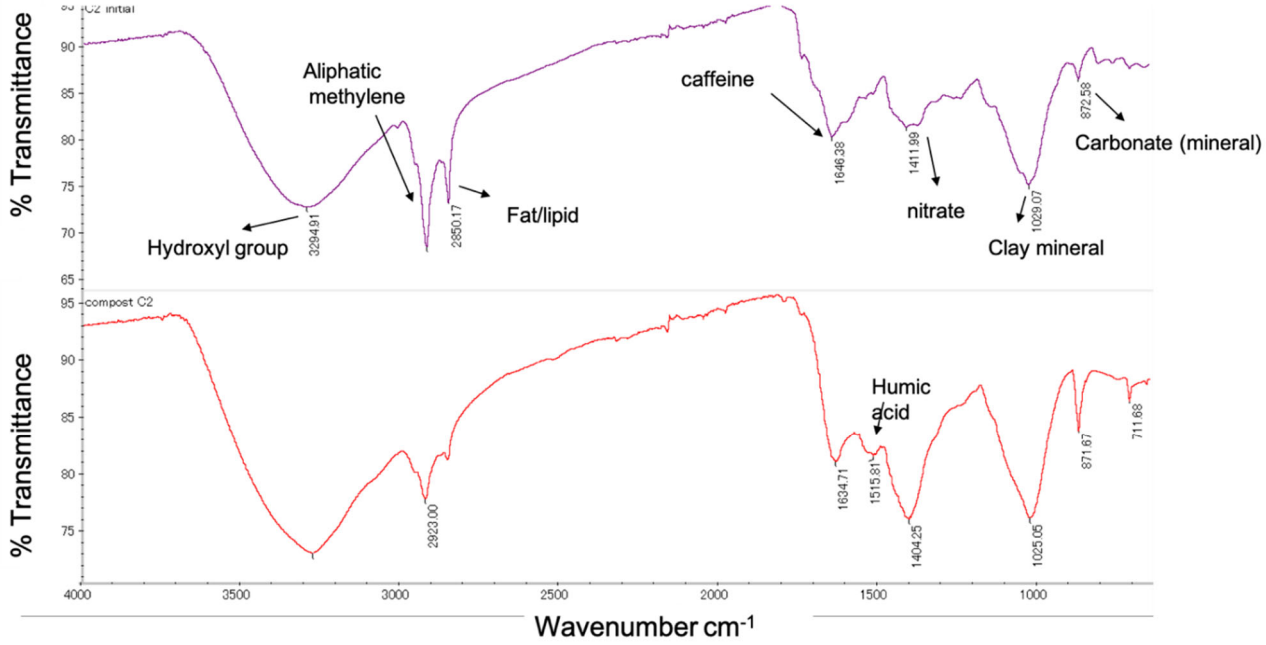


Figure 3.16. C2 initial (upper) and compost C2 (lower)

Compost control and C1 also have almost same functional group with C2 like Figure 3.16 above. However only a little bit different concentration. The results of the analysis using FTIR were then compared with previous studies. In the spectra of compost the absorption bands at 2850-2892 cm^{-1} are attributed to aliphatic methylene groups and assigned to fats and lipids. Lipids are an important fraction of compost that can influence the water retention capacity of amended soils, their structural stability and the biodegradation-humification balance in soils (Reveille et al., 2003). All three samples not have resources in that range, which means all four samples are mature and stable. While all sample initial have wavenumber 2849, 24.

The strong band at 1650 cm^{-1} can be assigned to amide I, carboxylates and C = C from aromatic and alkenes (Bohm, 2009). Of the four samples, C2 had slower peaks and lower wavenumber. This indicates that the aromatic structure is both lower than control and C1. Components rich in proteins of compost can be identified by a strong band between 1570 and 1540 cm^{-1} (Bohm, 2009). From Figure 18 above, all three samples, control, C1, C2, indicate reduced protein. Wavenumber 1515.57 at compost control is lignin. This is consistent with previous research by Kacurakova et al (2002), namely absorption bands in the region 1500- 1600 cm^{-1} could be assigned to the aromatic rings of lignin. Peaks at 1510, 1460, 1420, 1270, 1230, 1130 cm^{-1} are typical for lignin (Matias et al., 2000; Zancada et al., 2003). C1 and C2 not have this wavenumber, it is mean that compost C1 and C2 lignin degraded by microorganism.

In wavenumber 1384-1400 is a nitrate (Bohm, 2009). Sample C1 and C2 indicate the presence of a functional group N-O stretch which is nitrate. The intensity changes of bands at 1030-1040 testify the decomposition processes of organic components and can be used to evaluate the composting processes (Smidt et al., 2002). In another reference mentioned, in the region 1080-1010 cm^{-1} is assigned to C-O stretching of polysaccharides or polysaccharide-like substances, Si-O of silicate impurities, and clay minerals possible in a complex with humic acids (Filip and Bielek, 2000; Senesi et al., 2003).

Conclusion

Combination of some fungi activators with temperature control in composting SCG can improve quality compost produced, with the physical characteristics of compost black and crumb, and normal pH. While the chemical characteristics of compost produced is a C/N ratio below 10 with a far difference from the control. Compost is also richer in minerals, such as phosphorus, potassium, calcium, and magnesium, as well as rich in humic acid as shown from the results of the FTIR analysis. Addition of a combination of fungi starter such as *Aspergillus sp*, and *Penicillium sp* can compete with commercial activators. This is also evidenced from the results of the phytotoxicity analysis, where the Germination Index (GI) of the compost sample with the addition of fungi activator (C2) is 191.86% greater than the commercial activator (C1) 183.88%.

REFERENCES

Adegunloye, D.V., Adetuyi, F.C., Akinyosoye, F.A. and Doyeni, M.O. 2007. Microbial analysis of compost using cow dung as booster. Pakistan Journal of Nutrition 6, 506-510.

Adlini N.I. 2014. Cellulolytic microbial selection to degrade lignin from peat soil from Rimbo Panjang Village, Kampar Riau [Thesis]. Biology Department, Faculty of Mathematics and Natural Sciences, Riau University, Indonesia.

Agbede TM, Ojeniyi SO, Adeyemo AJ. 2008. Effect of poultry manure on soil physical and chemical properties, growth and grain yield of sorghum in Southwest Nigeria. Ame-Eurasian J. Sustainable Agric, 2(1), 72-77.

Ajayi, O.C. 2007. User Acceptability of Sustainable Soil Fertility Technologies: Lessons from Farmers' Knowledge, Attitude and Practice in Southern Africa. Journal of Sustainable Agriculture 30, 21-40.

Ali. 2004. Sustainable composting: Case studies and guidelines for developing countries, Water, Engineering and Development Centre, Loughborough University, Leicestershire.

Alvira P., Negro M.J., Ballesteros M. 2011. Effect of endoxylanase and α-Larabinofuranosidase supplementation on the enzymatic hydrolysis of steam exploded wheat straw. Bioresour Technol 102, 4552-4558. http:// doi: 10.1016/j.biortech.2010.12.112.

Ambriyanto, K. S. 2010. Isolation and Characterization of Aerobic Bacteria Cellulose Degradation from Leaf Litter Elephant (Pennisetum purpureum schaum). Indonesia, Biology Department. Faculty of Math and Science. Institute of Technology November 10, Surabaya, Indonesia.

Amir S, Hafidi M., Merlina G., Revel J C. 2005. Sequential extraction of heavy metals during composting of sewage sludge. Chemosphere, 59, 801-810. http://doi: 10.1016/j.chemosphere.2004.11.016

AOAC (Association of Official Agriculture Chemists). 2002. Official Methods of Analysis of AOAC International, Vol. 1, in Horwitz, W. (Eds.), Agricultural Chemicals, Contaminants, Drugs, AOAC International, 17th ed., Maryland, USA.

Atiyeh, R.M., Subler, S., Edwards, C.A., Bachman, G., Metzger, J.D. and Shuster, W. 2000b. Effects of vermicomposts and composts on plant growth in horticulture container media and soil. Pedobiologia, 44, 579–590.

Bargali, S.S. 2004. Cow Dung Burning is a Threat to Sustainable Agriculture. National Seminar on Ecology and Environment Management: Issues and Research Needs, Department of Botany, Kurukshetra Uniersity, Kurukshetra.

Bernabé G.A., Kobelnik M., Almeida S., Ribeiro C.A., Crespi M.S. 2013. Thermal behavior of lignin and cellulose from waste composting process. J. Thermal Anal. Calorim. 111, 589- 595. https://doi.org/10.1007/s10973-012-2276-8

Bernal M.P., Paredes C., Sanchez-Monedero M.A., Cegarra J. 1998. Maturity and stability parameters of composts prepared with a wide range of organic wastes. Bioresource Technology, 63, 91-99. https://doi.org/10.1016/S0960-8524(97)00084-9

Bikovens O., Dizhbite,T., Telysheva G. 2012. Characterization of humic substances formed during co-composting of grass and wood wastes with animal grease. Environ. Technol. 33, 1427-1433. http://doi:10.1080/09593330.2011.632652

Blanchette, R.A., Held, B.W., Jurgens, J.A., McNew, D.L., Harrington, T.C., Duncan, S.M., Farrell, R.L. 2004. Wood-destroying soft-rot fungi in the historic expedition huts of Antarctica. Applied and Environmental Microbiology, 70, 1328-1335. http://doi: 10.1128/AEM.70.3.1328-1335.2004

Bohm. 2009. Compost quality determination using infrared spectroscopy and multivariate data analysis. Wien: UNIVERSITÄT FÜR BODENKULTUR WIEN

Bosch Reig, F., Gimeno Atelantado, J.V., Moya Moreno, M.C.M. 2002. FTIR quantitative analysis of calcium carbonate (calcite) and silica (quartz) mixtures using the constant ratio method. Application to geological samples. Talanta 58 (4), 811–821.

Brouwer, J. and Powell, J.M. 1995. Soil aspects of nutrient cycling in manure experiment in Niger. In: Powell, J.M., FernandezRivera, S., Williams, T.O., Renard, C. (Eds.), Livestock and Sustainable Nutrient Cycling in Mixed Farming Systems of Sub-Saharan Africa. Technical Papers, Vol. II. Proceedings of an International Conference, 211–226.

Brouwer, J. and Powell, J.M. 1998. Micro-topography, water balance, millet yield and nutrient leaching in a manuring experiment on sandy soil in south-west Niger. In: Renard, G., Neef, A.,Becker, K., von Oppen, M. (Eds.), Soil Fertility Management in West African Land Use Systems. Proceedings of a Workshop, 349–360.

Caricasole P., Provenzano M.R., Hatcher P.G., Senesi N. 2011. Evolution of organic matter during composting of different organic wastes assessed by CPMAS C-13 NMR spectroscopy. Waste Manag, 31,411-415. http:// doi: 10.1016/j.biortech.2010.05.095.

CEN. 1999. European standards for waste sampling, Paris.

Chescheir PW, Westserman LM, Safley Jr LM. 1986. Laboratory methods for estimating available nitrogen in manures and sludges. Agric. Wastes 18, 175-195.

Cruz. 2014. A dissertation: Coffee by-Product, Sustainable Agro-Industrial Recovery and Impact on Vegetables Quality. Universidade do porto: Faculdade de Farmacia, Portugies.

Dinesh, R., Dubey, R.P., Ganeshamurthy, A.N. and Prasad, G.S. 2000. Organic manuring in ricebased cropping system: effects on soil microbial biomass and selected enzyme activities. Curruent Science 79(12), 1716-1720.

Duncan J. 2005. Composting chicken manure. WSU Cooperative Extension, King County Master Gardener and Cooperative Extension Livestock Advisor.

Echeveria, M.C., Cardelli, A., Bedini, S., Colombini, A., Incrocci, I., Castagna. 2012. Microbial Enhanced composting of wet olive husks. Bioresour. Technol. 104, 509-517.

Filip, Z., Bielek, P. 2002. Susceptibility of humic acids from soils with various contents of metals to microbial utilisation and transformation. Biol. Fertil. Soils 36, 426 – 433

Filip, Z., Pecher, W., Berthelin, J. 2000. Microbial utilization and transformation of humic acid-like substances extracted from a mixture of municipal refuse and sewage sludge disposed of in a landfill. Environmental Pollution 109, 83–89.

Fulhage, C.D. 2000. Reduce environmental problems with proper land application of animal manure. University of Missouri Extension. USA.

Fustec, E., Chauvet, E., Gas, G. 1989. Lignin degradation and humus formation in alluvial soils and sediments. Appl. Environ. Microbiol. 55, 922-926.

Gaind, S., Nain, L., Patel, V.B. 2009. Quality evaluation of co-composted wheat straw, poultry droppings and oil seed cakes. Biodegradation, 20, 307–317. http:// 10.1007/s10532-008-9223-1.

Gaschk, T. Ayaka, V. Hanh, Dominique Wisniewski. 2009. Waste Management in Devikulam. Sustainability in Developing Communities.

Gill, S.S., A.M. Jana and A. Shrivastav. 2014. Aerobic bacterial degradation of kitchen waste: A review. J. Microbiol. Biotechnol. Food Sci., 3(6), 477.

Gomes, T., Pereira, José A., Ramalhosa, E., Casal, S., Baptista, P. 2013. Effect of fresh and composted spent coffee grounds on lettuce growth, photosynthetic pigments and mineral composition. In VII Congreso Ibérico de Agroingenieria y Ciencias Horticolas. Madrid

Gupta, R., Mehta, G., Khasa, Y. P., Kuhad, R. C. 2011. Fungal delignification of lignocellulosic biomass improves the saccharification of cellulosics. Biodegradation, 22, 797–804.

Hachicha R., Rekik O., Hachicha S., Ferchichi M., Woodward S., Moncef N., Cegarra J., Mechichi T. 2012. Co-composting of spent coffee ground with olive mill wastewater sludge and poultry manure and effect of Trametes versicolor inoculation on the compost maturity. Chemosphere, 88, 677-682.

Haider K., Trojanowski J. 1975. Decomposition of specifically 14C-labelled phenol and

dehydropolymers of coniferyl alcohols as model for lignin degradation by soft- and white-rot fungi. Arch Microbiol, 105, 33–41.

Hand, P., Hayes, W.A., Frankland, J.C. and Satchell, J.E. 1988. Vermicomposting of cow slurry. Pedobiologia, 31, 199–209.

Haroun M., Idris A., Omar S. 2009. Analysis of heavy metals during composting of the tannery sludge using physicochemical and spectroscopic techniques. Journal of Hazardous Materials, 165, 111-119.

Hatakka A. 2001. Biodegradation of lignin. In: Hofrichter M, Steinbu¨chel A (eds) Biopolymers, vol 1: Lignin, humic substances and coal. Wiley-VCH, Weinheim, 129–180.

Hatmani A. 2000. Introducing of bacillus sp. Journal of Oseana, 25 (1), 31-41.

He X.S., Xi B.D., Jiang Y.H. 2013. Structural transformation study of water-extractable organic matter during the industrial composting of cattle manure. Microchem J, 106, 160- 166.

He, L., Zhao, X.L., Li, C.B. 2007. Impact of different packing on nitrogen transformation in composting process of municipal sewage sludge. Journal of Southwest China Normal University, 32(2), 54-58.

Henry, Chapter VII: Composting. 2005. Solid waste management. http://www.unep.or.jp/ietc/

Herpoel, I., Jeller, H., Fang, G., Petit-Conil, M. et al. 2002. Efficient enzymatic delignification of wheat straw pulp by a sequential xylanaselaccase mediator treatment. J. Pulp. Pap. Sci, 28, 3.

Horswill P, O'Sullivan O, Phoenix GK, Lee JA, Leake JR. 2007. Base cation depletion, eutrophication and acidification of species-rich grasslands in response to long-term.

Huang G.F., Wong J.W.C., Wu Q.T., Nagar BB. 2004. Effect of C/N on composting of pig manure with sawdust. Waste Management, 24, 805-813.

Inbar, Y., Chen, Y., Hadar, Y. 1989. Solid-state carbon-13 nuclear magnetic resonance and infrared spectroscopy of composted organic matter. Soil. Sci. Am. J, 53, 1695-1701.

Irawan B., R. S. Kasiamdari, B. H., Sunarminto and E., Sutariningsih. 2014. Preparation of Fungal Inoculum for Leaf Litter Composting From Selected Fungi. Journal of Agricultural and Biological Science. 9(3), 1-7.

Iwai H., Fukushima M., Yamamoto M., Komai T., Kawabe Y. 2013. Characterization of seawater extractable organic matter from bark compost by TMAH-py-GC/MS. J. Anal. Appl. Pyrol. 99, 9-15.

Joshi, R., Singh, J., Vig, A.P. 2015. Vermicompost as an effective organic fertilizer and

biocontrol agent: effect on growth, yield and quality of plants. – Reviews in Environmental Science and BioTechnology, 14(1), 137–159.

Justyna B. 2019. Changes in mineral forms of nitrogen and sulfur and enzymatic activities during composting of lignocellulosic waste and chicken feathers. Environmental Science and Pollution Research (2019) 26:10333–10342. https://doi.org/10.1007/s11356-019-04453-2.

K. Meissl, E. Smidt, J. Tintner. 2008. Reproducibility of FTIR spectra of compost, municipal solid waste and landfill material, Applied Spectroscopy 62, 190-196.

Kacurakova, M., Smith, A.C., Gidley, M.J., Wilson, R.H. 2002. Molecular interactions in bacterial cellulose composites studied by ID FT-IR and dynamic 2D FT-IR spectroscopy. Carbohydr. Res. 337, 1145 – 1153.

Kalab M. 2008. Conventional Scanning Electron Microscopy of Bacteria. Food Microstructure 3(1), 95-111.

Kasana, R.C., R. Salawan, H. Dhar, S. Dutt, A., Gulati. 2008. A rapid and easy method for the detection of microbial celluloses on agar plates using gram's iodine. Curr Microbiol., 57(5), 53-507.

Kerem, Z., Friesem, D., Hadar, Y. 1992. Lignocellulose degradation during solid-state fermentation: Pleurotuso streatus versus Phanerochaete chrysosporium. Appl. Environ. Microbiol, 58, 1121–1127.

Kesavan, P.C. and Swaminathan, M.S. 2008. Strategies and models for agricultural sustainability in developing Asian countries. Philosophical Transactions of the Royal Society B 363: 877–891. doi:10.1098/rstb.2007.2189.

Khan O. 2013. Soil: Principles, Properties, and Management. Bangladesh: University of Chittagong.

Kirk T.K., Farrell R.L. 1987. Enzymatic "combustion": the microbial degradation of lignin. Ann Rev Microbiol. 41, 465–505.

Kluczek-Turpeinen B., Tuomela M., Hatakka A., Hofrichter M. 2003. Lignin degradation in a compost environment by the deuteromycete Paecilomyces inflatus. Appl Microbiol Biotechnol, 61, 374–379.

Koji Yamane, Mitsuaki Kono, Taiji Fukunaga, Kazuya Iwai, Rie Sekine, Yoshinori Watanabe & Morio Iijima. 2014. Field Evaluation of Coffee Grounds Application for Crop Growth Enhancement, Weed Control, and Soil Improvement, Plant Production Science, 17(1), 93-102, http://doi: 10.1626/pps.17.93.

Kumari, P., Mathanker, G.K., Sharma, B. and Maurya, B.R. 2014. Effect of organic amendments on microbial population and enzyme activities of soil. Journal of Crop and Weed 10(1), 64-68.

Leifa F., Pandey A., R, Mohan., Soccol C.R., Mohan R. 2000. Coffee Biotechnology and

Quality. Journal of Basic Microbiology, 40 (3), 177-187.

Levin, L., Papinutti, L., Forchiassin, F. 2004.Evaluation of Argentine an white rot fungi for their ability to produce lignin-modifying enzymes and decolorize industrial dyes. Bioresour.Technol.94, 169–176.https://doi.org/10.1016/j.biortech.2003.12.002.

Li, L., Li, X. Z., Tang, W. Z., Zhao, J. et al. 2008. Screening of a fungus capable of powerful and selective delignification on wheat straw. Soc. Appl. Microbiol. Lett. Appl. Microbio, 47, 415–420.

Li, X., Pang, Y., Zhang, R. 2001. Compositional changes of cotton seed hull substrate during P. ostreatus growth and the effects on the feeding value of the spent substrate. Bioresour. Technol, 80, 157–161.

López- Masquera ME, Cabaleiro F, Sainz MS, López- Fabal A, Carral E. 2008. Fertilizing value of broiler litter: Effects of drying and pelletizing. Bioresource Technol. 99: 5626-5633.

Luz Cayuela M., Sanchez-Monedero M.A., Roig A., Sinicco T., Mondini, C. 2012. Biochemical changes and GHG emissions during composting of lignocellulosic residues with different N-rich by-products. Chemosphere 88,196-203.

Macrone MF. 2004. Composition and Properties of Indonesian Palm Civet Coffee (Kopi Luwak) and Ethiopian Civet Coffee. Department of Food Science, Ontario Agricultural College, Guelph, Ont., Canada N1G 2W1 (19 May 2004).

Madejova, J. 2003. FTIR techniques in clay mineral studies. Vibrational Spectroscopy, 31(1), 1-10.

Mandal, K.K., Rajak, A., Debnath, S.W. and Hasan, M.A. 2013. Integrated nutrient management in aonla cv A-7in the red laterite region of West Bengal. Journal of Crop and Weed 9: 121-23.

Marganingtyas, D.D. 2011. Potential of Indigenous Mangrove Cellulolytic Bacteria for Shrimp Pond Waste Composition. (Thesis) Faculty of Fisheries and Marine Sciences, University of Brawijaya, Malang, Indonesia.

Matias, M.C., Orden, M.U., Sanchez, C.G., Urreaga, J.M. 2000. Comparative spectroscopic study of the modification of cellulose materials with different coupling agents. J. Appl. Polym. Sci. 75, 256 – 266.

Milstein, O.A., Haars A., Sharma A., Vered Y. 1984. Lignin Degrading Ability of Selected Aspergillus spp. Jerman, lnstitut der Universitat Gottingen, FRG. The Humana Press Inc.

Mishra, V. And Prasad, D.N. 2005. Application of in vitro methods for selection of *Lactobacillus casei* strains as potential probiotics. International Journal of Food Microbiology. 103, 109- 11.

Misra R.V., Roy R.N. 2003. On Farm Composting Methods. A book: land and water discussion.

http://www.fao.org/3/y5104e/y5104e00 [1 December 2020]

Mistia, S. 2012. Aplication of Probiotic Bacteria: Pediococcus pentosaceus and cow dung for composting cacao leaf waste. Masters thesis, Andalas University, Indonesia.

Morisaki N., Phae C.G., Naksaki K., Shoda M., Kubota H. 1989. Nitrogen Transformation during thermophilic composting. Journal of Fermentation and Bioengineering, 67, 57-61.

Murbandono, L. 2003. Membuat Kompos. Penebar Swadaya: Jakarta, Indonesia.

Murthy, P. S., & Naidu, M. M. 2012a. Production and application of xylanase from *Penicillium sp.* utilizing coffee by-products. Food and Bioprocess Technology, 5(2), 657-664. http://dx.doi.org/10.1007/ s11947-010-0331-7.

Paradelo R., Moldes A.B., Barral M.T. 2013. Evolution of organic matter during the mesophilic composting of lignocellulosic winery wastes. J. Environ. Manag. 116, 18-26.

Pichtel, J.R. 1990. Microbial respiration in fly ash-sewage sludge amended soils. Environmental Pollution, 63(3), 225-227. http://doi:10.1016/0269-7491(90)90156-7.

Prasad P, Power JF. 1997. Soil fertility management for sustainable agriculture. Lewis Publishers, Boca Ratoni.

Re´veille´, V., Mansuy, L., Jarde´, E., Garnier-Sillam, E. 2003. Characterization of sewage sludge-derived organic matter: lipids and humic acids. Organic Geochemistry 34, 615-627.

Rebollido. Martinez J. 2008. Microbial population during composting process of organic fraction of municipal solid waste. Applied Ecology and Environmental Research, 6(3), 61-67.

Regalado V., Rodrı´guez A., Perestelo F., Carnicero A., De La Fuente G, Falcon MA. 1997. Lignin degradation and modification by the soil-inhabiting fungus *Fusarium proliferatum.* Appl Environ Microbiol, 63, 3716–3718.

Riffaldi R., Leviminzi R., Pera A., Debertoldi M. 1986. Evaluation of compost maturity by means of chemical and microbial analyses. Waste Management & Research, 4, 387-396.

Rodriguez A., Perestelo F., Carnicero A., Regalado V., Perez R., De La Fuente G., Falcon MA. 1996. Degradation of natural lignins and lignocellulosic substrates by soil-inhabiting fungi imperfecti. FEMS Microbiol Ecol 21, 213–219.

Ros M., Pascual J.A., Garcia C., Hernandez M.T., Insam H. 2006b. Hydrolase activities, Microbial biomass and bacterial community in a soil after long-term amendment with different composts, Soil Biol. Biochem. 38, 3443–3452.

Shahriarinour, M., M.N.A., Wahab, A., Ariff and R. Mohamad. 2011. Screening, isolation and selection of cellulosic fungi from oil palm empty fruit bunch fibre. Biotechnol, 10, 108- 11.

Singh A, Shahid M, Srivastava M, Pandey S, Sharma A, et al. 2014. Optimal Physical Parameters for Growth of *Trichoderma* Species at Varying pH, Temperature and Agitation. Virol Mycol 3: 127. http://doi:10.4172/2161-0517.1000127

Smidt, E., Lechner, P., Schwanninger, M., Haberhauer, G., Gerzabek, M.H. 2002. Characterization of waste organic matter by FTIR spectroscopy – application in waste science. Applied Spectroscopy 56, 1170–1175.

Smith, B. 1999. Infrared Spectral Interpretation. CRC Press, Boca Raton, London, New York, Washington; DC.

Socrates, G. 2001. Infrared and Raman Characteristic Group Frequencies, third ed.Tables and Charts John Wiley and Sons Ltd., Chichester

Sreedevi, S., S. Sajith and S. Benjamin. 2013. Cellulase producing Bacteria from the wood-yards on Kallai river bank, Adv. Microbiol, 3, 326-332.

Stephenson AH, McCaskey AT, Ruffin BG. 1990. A Survey of broiler litter composition and potential value as a nutrient resource. Biol. Wastes, 34: 1-9.

Subowo Y.B. 2015. Testing the activity of *Penicillium sp.* R7, 5 and *Aspergillus niger* NK on growing media to support the growth of rice plants in saline land. Pros Sem Nas Masy Biodiv Indonesia, 1(5), 1136-1141.

Subowo YB, Corazon. 2010. Selection of Lignin and PAH Decomposed Soil Fungi from Several Environments in Bali. Biology News, 10(2), 227-234.

Tuomela M., Vikman M., Hatakka A., Ita¨vaara M. 2000. Biodegradation of lignin in a compost environment: a review. Bioresour Technol, 72,169–183.

United States Composting Council. 2008. Greenhouse gases and the role of composting: A primer for compost producers. http://www.compostingcouncil.org

Varadachari, V., Ghosh, K. 1984. On humus formation. J Plant Soil, 77, 305-313.

Viikari, L., Kantelinen, A., Sundquist, J., Linko, M. 1994. Xylanases in bleaching: from an idea to the industry. FEMS Microbiol. Rev. 1994, 13, 335–350.

Wang L.P., Shen Q.R., Yu G.H., Ran W., Xu Y.C. 2012a. Fate of biopolymers during rapeseed meal and wheat bran composting as studied by two-dimensional correlation spectroscopy in combination with multiple fluorescence labeling techniques. Bioresour. Technol. 105, 88-94.

Warman PR. 1986. The effect of fertilizer, chicken manure and dairy manure on Timothy yield, tissue composition and soil fertility. Agric. Wastes 18, 289-298.

Watanabe. 2002. Pictorial Atlas of Soil and Seed Fungi Morphologies of Cultured Fungi and Key to Species, Second Edition. Boca raton: CRC press. https://doi.org/10.1201/9781420040821.

Williams, T.O., Powell, J.M. and Fernandez-Rivera, S. 1995. Manure availability in relation to sustainable food crop production in semi-arid West Africa: evidence from Niger. Quarterly Journal of International Agriculture 34(3), 248–258.

Wong MH, Cheung YH, Cheung CL. 1983. The effects of ammonia and ethylene oxide in animal manure and sludge on the seed germination and root elongation of Brassica parachinensis (flowering Chinese cabbage). Environmental Pollution Series A. Ecol. Biol. 30: 109-123.

Yuleli. 2009. The Use of Several Types of Fungi to Increase Growth of Rubber Plants (Hevea brasiliensis) in Peatlands [Thesis]. Medan: University of North Sumatra, Indonesia.

Zancada, M.C., Almendros, G., Ballesta, R.J. 2003. Humus quality after eucalypti reforestations in Asturias (Northern Spain). Sci. Total Environ. 313, 245 – 258.

Zeng, J., G.W. Price and P. Arnold. 2012. Evaluation of an aerobic composting process for the management of Specified Risk Materials (SRM). J. Hazard Mater. 219, 260-266.

Zhao L, Gu WM, He PJ, Shao LM. 2011a. Biodegradation potential of bulking agents used in sludge bio-drying and their contribution to bio-generated heat. Water Res. 45, 2322-2330.

CHAPTER 4

Implementation of SCG compost On Mustard Plant (Brassica Juncea L)

Chapter 4

4.1. Background Research

Spent Coffee Ground (SCG) compost is a compost made with raw materials SCG, cow dung, and chicken manure, with the addition of *Aspergillus* sp. and *Penicillium* sp. starters. The nutrients contained in the SCG compost are very good when implemented in horticultural crops. Compost SCG has a C / N ratio below 10, normal pH and is rich in minerals, such as potassium, phosphorus, calcium and magnesium (Afriliana et al., 2020). These elements are used in relatively large amounts by the plant and are called macronutrients. Using compost particularly, in intensive industries such as vegetable production, has demonstrated potential to reduce the need of fertilizer, irrigation and pesticides, and to improve marketable yields (Paulin and Malley, 2008). It also can improve soil fertility, water holding capacity, organic matter content, and ultimate crop yields, improve product quality, and extend shelf life (Stofella et al., 2014).

In the final compost product, the number of microorganisms, fungi in the compost SCG that we previously made was still high, around 6.94 log cfu/ml. This could be an advantage or potential of SCG compost. In previous research, enrichment of compost in terms of increasing the nutrient content of final compost product had been studied (Shinde et al, 1990a; Bhanawase Hajra et al., 1994; Zayed and Abdel-Motaal, 2005; Gaind et al., 2006). Microbial enrichment technique with bio-inoculants to composting material had been shown to improve the quality of compost (Gaur, 1982, Rasal Shinde et al., 1990b; Arora and Garg, 1992; Arora et al, 1994) and even in low-grade city compost (Talashilkar, 1985). Further, the most of the work relating to compost enrichment is done during the composting period.

Aspergillus and *Penicillium* are types of fungi that can convert insoluble phosphate into soluble phosphate or what is called Phosphate solubilizing fungi (PSF). They produce low molecular mass organic acids (Deubel and Merbach, 2005), which attack the phosphate structure and transform phosphorus from non-utilizable to the utilizable form for the plants (Ivanova et al., 2006). PSF are able to improve the phosphorus nutrition and stimulate the plant growth. Solubilization is due to the secretion of organic acids by microorganisms which reduces the pH or by complexing cation that is bound to phosphorus, in turn decreasing the particle size. However, the role of organic acids and low pH, holds a major role to play in phosphate availability in vitro and in vivo (Kim et al., 1997).

The PSF-rich SCG compost can also act as Plant Growth promoting Fungi (PGPF). PGPF are heterogeneous groups of non-pathogenic saprotroph fungi. They are the group of rhizosphere fungi that colonize plant roots and enhance plant growth. Over the decades, varieties of PGPF have been studied including those belonging to genera *Penicillium, Aspergillus, Trichoderma, Phoma* and *Fusarium* (Hossain et al., 2020). Studies have shown that PGPF modulates plant growth and enhances resilience to plant pathogens without environmental contamination (Hossain et al., 2017). The positive effects of PGPF on plants and the environment make them well fitted to organic agriculture.

Therefore, it is necessary to study the benefits of SCG compost in plants. One of the horticultural crops that is widely cultivated by farmers is mustard greens. According to Inonu et al (2014), mustard greens are a type of vegetable with high economic value. In addition, the harvest age is relatively short, namely 40-50 days after planting. Demand for mustard plants always increases with increasing population and awareness of nutritional needs (Haryanto et al., 2006). PGPF exhibit traits beneficial to plants and as such, their capacity to enhance plant growth and development is well founded. PGPF mediates both short and long term effects on germination and subsequent plant performance. Improvement in germination, seedling vigor, shoot growth, root growth, photosynthetic efficiency, and yield are the most common effects decreed by PGPF. A particular PGPF may condition plant growth by exerting all or one or more of these effects (Hossain et al., 2020).

Therefore, in this study the objective was to study the effect of implementing SCG compost rich PGPF on the growth of mustard plants both physically and the nutritional content of plants under greenhouse conditions.

4.2.Literary Review

4.2.1. SCG Compost

In the previous chapter, we learned about composting Spent Coffee Grounds (SCGs) with the addition of other raw materials such as cow dung and chicken manure. After composting with a commercial starter and fungi, compost is produced with a high germination index and nutrients needed by plants. Previously, researchers had also conducted research on SCG compost as a growing media for production of Brassica seedling (Chrysargyris., 2020), for production of bacilli and tomato plants (Ronga et al., 2015). Of course, the method of composting and the type of material used by each researcher is different. However, they concluded that SCG compost is very suitable to be applied to these types of plants, although each plant certainly has its own nutritional needs, so the percentage of added SCG compost in each of these plants is also different.

The SCG compost that we produce has the following nutrients or nutrients: C / N ratio around 7/1, phosphorus around 3-5%, nitrogen around 2-3%, and germination index around 180-190%. Phosphate is the main anion form which provides the element phosphorus (P) to plants. Phosphate ions are more easily absorbed by plants and are very vital because they are a major part of the plant genetic material framework (DNA and RNA) and become energy carriers for ATP and NADP compounds. While, nitrate is a major source of N for plant growth, especially for vegetables, particularly under aerobic agricultural conditions. Plants absorb nitrogen from the soil in the form of nitrate (NO_3^-) and ammonium (NH_4^+). In aerobic soil while nitrification can occur, nitrate is usually the predominant form of available nitrogen that is absorbed (Xu et al., 2012).

In the final compost product, the number of microorganisms, fungi in the compost SCG that we previously made was still high, around 6.94 log cfu/ml. This could be an advantage or potential of SCG compost, because it is rich in PGPF (Plant Growth promoting Fungi) (Afriliana et al., 2021).

4.2.2. Compost Enrichment

Application of compost to agricultural land has proven significantly beneficial to soil quality, crop yield and quality. Compost has been shown to improve soil texture and aggregation as a result of microbial contribution of polysaccharides, humic acid and other organic matter. Compost also enhances nutrient cycling through increased microbial activity and contributes for plant nutrition (Raviv, 2005). Compost increases soil water holding

capacity and improves water infiltration rates in soils (Buckerfield and Webster, 1998). In intensive cropping systems, supplementing soil nutrients by the use of chemical fertilizer is considered inevitable for obtaining optimum yield of crops. But it has been observed that continuous use of chemical fertilizers may affect soil health and may lead to a negative impact on soil productivity.

The preparation of quality compost within a short period of time during its mass requirement is another real challenge. Application of efficient decomposer microbial culture to compost raw materials can greatly increase the speed of composting and can improve the rate of nutrient release from compost after its addition into soil (Haseena, 2016). Inoculation of compost with bio-fertilizer/ bio protectant group of microorganisms enriches compost quality. Addition of different natural organic sources such as wood ash and rock phosphate would satisfy the immediate and long term nutrient requirement of different crop plants. Previous researchers (Liu et al., 2017; Pan et al., 2009) have reported that the application of organic fertilizer and chemical fertilizer can improve microbial activity, biomass, and nutrient utilization efficiency compared with the application of chemical fertilizers only.

Gaur (1982) studied the effect of mesophilic cellulolytic fungi on composting and reported that due to inoculation, the period of composting was reduced by one month and the quality of compost improved. Total nitrogen, phosphorous and humus contents increased in composted material and inoculation with *Aspergillus niger* and *Penicillium sp.* showed maximum effect.

Hegarty and Curran (1985) showed that different fungi used in their study produced greater weight loss of wood in 5 to 8 pH range. Inbar et al. (1988) found that organic matter with a wide range of pH (3.0 to 11.0) can be composted. The optimum pH levels were between 6 and 8 for composting and between 4 and 7 for end products.

4.2.3. Plant Growth promotion Fungi (PGPF)

Plants have intricate relationships with an array of microorganisms, particularly rhizosphere fungi and bacteria, which can lead to an increase in plant vigor, growth and development as well as changes in plant metabolism (Aly et al., 2014). The group of rhizosphere fungi that colonize plant roots and enhance plant growth is referred to as PGPF (Hyakumachi, 1994). PGPF are heterogeneous groups of non-pathogenic saprotroph fungi. They can be separated into endophytic, whereby they live inside roots and exchange metabolites with plants directly, and epiphytic, whereby they live freely on the root surface and

free-living PGPF, which live outside plant cells, i.e., in the rhizosphere (Hossain et al., 2017). PGPF establishes a non-obligate mutualism with a broader range of host plants. That is why symbiotic mycorrhizal fungi are not considered as PGPF, although they are known to improve growth of the plants (Bent, 2006).

Moreover, PGPF encompasses a diverse taxonomic group in comparison to mycorrhiza. They are often involved in a range of complex interactions with plants and develop distinct strategies to mediate improvements in seed germination, seedling vigor, plant growth, flowering and productivity of host plants. PGPF are not only associated with the root to mediate positive effects on plant growth and development but also have beneficial effects on suppressing phytopathogenic microorganisms (Hossain, 2007). Not every organism identified as PGPF will improve plant growth under all conditions or in association with all plant hosts (Hyakumachi et al., 2004). Some PGPF biocontrol inoculants usually contain necrotrophic mycoparasites such as *Trichoderma spp*. (Kaewchai et al., 2009), while a limited number such as Sphaeroides mycoparasitica is biotrophic mycoparasitic agent (Vujanovic et al., 2012). Therefore, PGPF is considered one of the potential active ingredients in both bio fertilizer and mycofungicide formulation.

PGPF exhibit traits beneficial to plants and as such, their capacity to enhance plant growth and development is well founded. PGPF mediates both short- and long-term effects on germination and subsequent plant performance. Improvement in germination, seedling vigor, shoot growth, root growth, photosynthetic efficiency, flowering, and yield are the most common effects decreed by PGPF.

4.2.4. Macronutrient for Plant Growth

Macronutrients are those elements needed by plants in the greatest quantities. Three of these are always represented on fertilizer packages as the N-P-K trilogy: nitrogen (N), phosphorus (P), and Potassium (K). Beyond this trilogy, other nutrients such as sulphur, calcium, and magnesium. These are also used by plants in large amounts. Carbon, hydrogen, and oxygen are also macronutrients (Lowenfels, 2013). The following is an explanation of the functions of each macronutrients:

a. Nitrogen (N)

Nitrogen is crucial for plant growth. One could argue, its presence as the backbone of amino acids, the structural building blocks of protein, one of the four kinds of molecules that make up life. It means that no nitrogen, no protein.

Enzymes are protein. Photosynthesis and respiration absolutely require nitrogen and the enzymes necessary to drive these processes. Proteins are also the molecules that make cellular membranes semi-permeable. They are the channels, carriers, and motors that are necessary for sufficient quantities of water and any quantity of other essential nutrients to get into a plant cell.

Nitrogen also has a role as the base element for nucleotide molecules. These are the building blocks of DNA and RNA, the blueprints and translators, respectively of genetic code. Besides, nitrogen is also an essential part of the chlorophyll molecule ($C_{55}H_{72}MgN_4O_5$). Without those four nitrogen atoms, here is no photosynthesis. Therein, incidentally, lies the answer to the yellowing lawn: a lack of nitrogen means there is less chlorophylls green pigment.

Plants take up N from the soil as NH_4^+ (ammonium) or NO_3^- (nitrate). A typical plant contains 1.5 percent nitrogen on a dry weight basis, but this can range from 0.5 percent for a woody plant to up to 5.0 percent for a legume (Mahler, 2004). Outside of the plants itself, nitrogen also has a great influence on the pH of the soil, which has direct influence on the uptake of nutrients. The pH in the Rhizosphere goes up when NO_3 is added because hydroxyl ions (OH^-) are released. This increases the solubility of iron and aluminium phosphates.

Making nitrogen usable by plants is nitrogen fixation. Fixed nitrogen in the form of ammonium (NH_4^+) and nitrate (NO_3^-) is useful as nutrient to plants and is taken up and assimilated into various compounds within the plant.

b. Phosphorus (P)

Next to nitrogen, phosphorus (P) is the second most important macronutrient as an essential plant nutrient (Srinivasan et al., 2012). It is a key nutrient for higher and sustained agricultural productivity (Scervino et al., 2011) and which limits plant growth in many soils. Phosphorus forms an important component of the organic compound adenosine triphosphate (ATP), which is the energy currency that drives all biochemical processes in plants (Brady et al., 2008). It is also an integral component of nucleic acids, coenzymes, nucleotides, phosphoproteins, phospholipids and sugar phosphates as well as intermediates of signal transduction events (Khan et al., 2010; Plaxton et al., 2015; Razaq et al., 2017). It is also involved in an array of processes in plants such as photosynthesis, respiration, nitrogen fixation, flowering, fruiting, and maturation (Brady et al., 2008; Plaxton et al., 2015; Xiang et al., 2012) Plant dry matter may contain up to 0.5% phosphorus (Brady et al., 2008).

Despite the important role played by soil P in plants, however, phosphorus deficiency in soil is the most common nutritional stress in many regions of the world, affecting 42% of

the cultivated land in the world (Liu et al., 1994). The P deficiency is caused either by low P content in the soils parent materials or by transformations of P.

Phosphorus ultimately comes from the weathering of apatite, a type of rock. It can enter soils into two forms: organic and inorganic. Inorganic phosphorus, from apatite, is adsorbed to the surface of clay particles and organic matter. The attraction is an ionic one, binding the phosphorus in place, and as a result it does not move much in soil.

Inorganic phosphorus (Pi) occurs mainly as $H_2PO_4^-$ and HPO_4^{2-} adsorbed onto the surfaces of oxides and hydroxides of Fe or Al, organic matter or bound to Ca (Olibone et al., 2010). The adsorption of P in soils depends on soil pH and clay minerals (Duputel et al., 2013). However, most Pi occurs in the clay fraction as salts of orthophosphoric acid. Phosphorus forms insoluble compounds with iron and aluminium at low pH, more soluble compounds with calcium and magnesium at pH values near neutrality, and insoluble compounds with calcium at higher pH values (Brady et al., 2008; Tisdale et al., 1985; Shen et al., 2011). There is a wide range of solubility of these various phosphate compounds and their availability to crops is optimal within the soil pH range of about 6.0 to 6.5 for most agricultural soils. The Pi compounds are grouped as calcium or magnesium-bound and iron and aluminium-bound (Brady et al., 2008).

c. Potassium (K)

Potassium (K) is the most abundant inorganic cation, and it is important for ensuring optimal plant growth (White and Karley, 2010). K is an activator of dozens of important enzymes, such as protein synthesis, sugar transport, N and C metabolism, and photosynthesis. It plays an important role in the formation of yield and quality improvement (Marschner, 2012; Oosterhuis et al., 2014). K is also very important for cell growth, which is an important process for the function and development of plants (Hepler et al., 2001).

In terms of the growth-promoting mechanism of K, it is generally agreed that K stimulates and controls ATPase in the plasma membrane to generate acid stimulation, which then triggers cell wall loosening and hydrolase activation (Oosterhuis et al., 2014), thus promoting cell growth.

K has strong mobility in plants and plays an important role in regulating cell osmotic pressure and balancing the cations and anions in the cytoplasm (Kaiser, 1982; Hu et al., 2016a).Through these processes, K is involved in the regulation of stomatal opening and closing, cell elongation, and other important physiological processes.

There are many studies on the effect of K level on plant growth. Jin et al. (2007) found that the highest yield and fruit quality were obtained in Red Fuji apple under treatment with 600 kg/ha K; Wang et al. (2017) determined that 6 mM K treatment promoted pear growth and improved photosynthetic efficiency; and Lu et al. (2001) also reported increased production with better fruit quality parameters in navel orange supplied under 500 kg/ha K. There is an interaction between K and other nutrient ions. High K concentrations in the soil solution inhibit Mg uptake and may induce Mg deficiency in plants (Trankner et al., 2018). However, K deficiency could promote the absorption of Na^+ and Ca_2^+ in maize (Du et al., 2017), and could inhibit N absorption in cotton and significantly reduce the content of NO_3^- in the leaves (Hu et al., 2017). It is evident that K affects significantly the absorption and utilization of other nutrients by plants, and the appropriate K level differs in different crops.

Among the interactions between K and other nutrients, the interaction with N is the most important. Some studies evaluated the relationship between K and N metabolism. In contrast to the antagonistic relationship between K^+ and NH_4^+ nutrition, the acquisition rates of K^+ and NO_3^- are often found to be positively correlated (Rufty et al., 1981; Coskun et al., 2016), and sufficient K supply can promote N metabolism and enhance the synthesis of amino acids and proteins (Ruan et al., 1998; Ruiz and Romero, 2002). Hu et al. (2016b) found that K deficiency could reduce Nitrate reductase (NR), Glutamine synthetase (GS), and Glutamate synthase (GOGAT) activities and inhibit nitrate absorption in cotton, whereas Armengaud et al. (2009) found that K deficiency could up-regulate the activities of GS and Glu dehydrogenase (GDH) in Arabidopsis.

Metabolism of N affected by K appears to vary in different types of plants. Meanwhile, the level of K has a significant impact on C metabolism, and also a strong interaction exists between C metabolism and N metabolism in the metabolic process and energy level (Hu et al., 2017). Based on previous studies, we further evaluated the effects of different K levels on photosynthesis, C metabolism, nitrate uptake, utilization and distribution of M9T337 seedlings through non-invasive micro-measurement technology, ^{15}N, ^{13}C isotope labelling, and fluorescence quantitative PCR technology. Our studies provided strong evidence for the direct or indirect impact of K level on N absorption and utilization. These experimental results will provide a scientific basis toward the amelioration of problems, related to the poor growth and low N utilization rate of M9T337 seedlings caused by unreasonable K application.

d. Calcium (Ca)

Calcium is a key structural of cell walls. Because they form around every plant cell, this element is of immediate and obvious importance. Calcium is also a part of many enzymes and it is important as a signalling molecule. Under normal cell condition, the cytosol has a very low calcium concentration, with supplies sequestered in various organelles, such as the vacuole or endoplasmic reticulum. Increasing concentrations of calcium activate enzymes in the cytosol, speeding up reactions and causing things to happen (Lowenfels, 2013).

Calcium is also used to transport other substances across the cellular membrane. Because it is also important to cell division, it is not surprising that calcium is found in large concentrations in young undifferentiated meristematic cells that are dividing. This is why calcium deficiencies appear first in the growing areas of roots, shoots, and young leaves and the result of the deficiency is that they become malformed. Calcium is not mobile once assimilated into a cell. It is extremely abundant in the environment, and it comes from weathered minerals, such as limestone and chalk.

e. Magnesium (Mg)

The key function of magnesium (Mg) is its role as the centre of the chlorophyll molecule ($C_{55}H_{72}N_4O_5$). It is easy to see why this element is essential to plants: No magnesium, no photosynthesis. Magnesium is also an activator of enzymes involved in the production and use of ATP, so it plays a key role in respiration as well. Finally, magnesium is needed for the creation of DNA and RNA.

When plants develop a magnesium deficiency, chlorophyll cannot be synthesized and photosynthesis ceases. Leaves start to lose their green color in between the leaf veins, which are nearest the remaining sources of sugars, whereas the veins remain green, a condition known as interveinal chlorosis. Older leaves show the sign of magnesium deficiency first, meaning that magnesium is mobile in plants. Magnesium is a very abundant mineral on earth, and it weathers out of many minerals. The most familiar form is dolomite ($CaMg[CO_3]_2$), a sedimentary carbonate rock. Much magnesium is dissolved in water.

4.2.5. **Mustard plant**

Brassicaceae comprise a diverse family of plants and provide one of the most extensive and varied range of end products used by man from a single plant genus. Mustards are members of the Brassicaceae family, and are among the earliest cultivated plants. Their seeds are one of

the oldest recorded spices with use and cultivation dating back over 5000 years (Campbell et al., 2016; Watson et al., 2010). Mustard plants can be seen in Figure 4.1.

Figure 4.1. Mustard Plant (Indonesian Ministry of Agriculture, 2020)

Known for its impressive nutritional properties, Japanese mustard plant or komatsuna is chock full of vitamin C, calcium, and beta carotene. Like the other members of the brassica family, komatsuna has a compound called sulforaphane that helps our bodies fight cancer. Sulforaphane actively kills cancer stem cells, slowing a tumor's growth. Komatsuna, which has the scientific name Brassica rapa var. perviridis which belongs to the Brassica family, is a green, leafy vegetable that is sometimes called Japanese mustard spinach, tendergreen, or mizuna. It is endemic to Korea, Vietnam, Japan, and Taiwan. It got its name from Japan's Komatsugawa district and is considered a staple in Japanese cuisine.

Mustards have been consumed for centuries as vegetables, and their products used as condiments and as edible and industrial oils (Raymer et al., 2002; Nesi et al., 2016). The oil is commonly used for cooking and to add a hot and spicy flavor to food (Duke, 2002). As a crop, they are also one of the highest oil yielding and high protein containing oilseed species. Economically important members of this family include vegetables like broccoli, cabbage, Chinese cabbage, turnip, and cauliflower, and the seed oil crop canola (Collett et al., 2014; Spragg et al., 2016).

The optimum rate of fertilizer for organic cultivation of leaf-mustard ranged from 34 to 51 t/ha processed poultry manure depending on the cultivar used. Yields obtained ranged from 8 to 15 t/ha. For the second crop, residual fertilizer gave 60–70% of the yield of fertilizer applied plots, indicating the possibility of reducing the rate of organic fertilizer for subsequent crops (Vimala et al., 2010).

A valuable part of the mustard plant economically is the leaves hence effort increased production is attempted on increase in vegetative products to support these efforts carried out fertilization. Mustard plants require sufficient and available nutrients for growth and development to produce maximum production. Wrong a nutrient that is very important to leaf growth is Nitrogen. Nitrogen this serves to improve vegetative growth, thus leaves the plant becomes wider, more colourful green and higher quality (Wahyudi, 2010).

4.2.6. Plant Biomass and Nutrient Uptake

Lodhiyal et al. (2003) stated that in general, biomass is the total organic material content of a living organism at a certain time and place. Whittaker et al. (1975) stated that plant biomass is the dry material of a living organism (plant) at a certain time, place and area, therefore plant biomass units are usually expressed in kg/m^2 or tonnes/ha. The plant biomass in is expressed in terms of dry weight, which is a combination of the living plant organs above the ground (total aboveground biomass), whose main components consist of stem, branches / twigs and leaves.

Measurement of tree biomass can be done in various ways, for example by Brown et al. (1984) through an approach to calculating tree volume and wood density and to obtain total biomass. In one whole tree need to be multiplied by the number BEF (Biomass Expansion Factor). Ogawa and Kira (1977) stated that the measurement of the biomass of a tree can be done by using the total harvesting method, meaning that all tree components are harvested in an area with a certain area. In addition, tree biomass can also be calculated using the allometric method, namely harvesting several tree samples in a destructive way, weighing the wet weight and dry weight of the sample of each plant organ, then an equation is created which states the relationship between the dependent variable in the form of total biomass (Bt) and the independent variables can be stem diameter (D), tree height (H), wood density (ñ), or a combination of these variables.

Besides that, the weight of fresh stew is also closely related to nutrient and water uptake. This is in accordance with the opinion of Sarief (1986) which states that sufficient nutrients are available when plant growth results in photosynthesis running more actively, thus the process of cell elongation, division and differentiation will occur better which can support plant growth. The addition of fresh weight is also influenced by the availability of sufficient and balanced nutrients because this will increase cell division so that it becomes better and fresh weight is also influenced by the water content in plants (Goldworthy and Fisher, 1992).

According to Harjadi (1984), plant growth is a function of its efficiency in producing plant dry matter. Dry weight is closely related to increased growth and development in absorbing nutrients for growth and development of the vegetative part. If the dry weight is low, the vegetative growth of the plant will be hampered, because the nutrients are absorbed a little so that it affects plant growth. Plant dry weight reflects nutrient status and the amount of nutrients absorbed by plants and the rate of photosynthesis. Nutrients in plants play a role in the process of plant metabolism to produce dry matter which depends on the rate of

photosynthesis. If the rate of photosynthesis is different, the amount of photosynthate produced is also different, as well as the dry weight of the plant which is a reflection of the plant growth rate (Dwijoseputro, 1992). Prawiranata et al. (1988) stated that the dry weight of a plant is the result of the accumulation of photosynthate, which in its formation requires nutrients, water, CO2 and sunlight. This condition is supported by the opinion of Lakit (2004) which states that plant dry weight reflects the accumulation of organic compounds which are the result of plant synthesis from inorganic compounds derived from water and carbon dioxide so that they contribute to plant dry weight.

4.3.Material and Method

4.3.1. Material

This experiment was carried out in the greenhouse Faculty of Life and Environmental Science, Prefectural University of Hiroshima Japan. Soil was used are commercial black pure soil (Merck Gex, Japan) to fill plastic mini pot. Before used, Soil was analysed for chemical properties. Size of plastic mini pot is diameter around 8 cm, with height 7 cm. while source of compost were from SCG compost with 3 typical treatments: without starter (C0), with commercial starter (C1) (*Bacillus* starter from Industrial technology centre, Fukuoka Prefectural, Japan), with fungi starter (C2). Besides, we also used commercial compost (production Juntendo, Shimane, Japan) for comparation. Kind of plant we used are Mustard Plants (varieties 34881, Utsunomiya, Tohoku, Japan).

Meanwhile, the chemical materials used are, nitric acid, HCl, sulfuric acid, hexaammonium peroxodisulfate, acetic acid. The tools used are macro corder (JM 1000CN), AAS AA-6300, oven, blender, SEM (Scanning Electron Microscopy) JSM-7900F Schottky field emission and greenhouse. Greenhouse setting: every pot put on greenhouse completed by light from yellow LED, and temperature control 30^0 Celsius.

4.3.2. Method

4.3.2.1.Sample Preparation

The concentration of the compost added to each pot was 1, 2, 3% of the total soil. The amount of soil used in each polybag is 50 gr. Here are the details for samples.

1. S: soil
2. C0: compost control
3. C1: SCG compost with adding commercial starter

4. C2: SCG compost with adding fungi starter
5. P0: plant without adding compost
6. PC0 1%: plant with adding sample C0 1% of amount soil
7. PC0 2%: plant with adding sample C0 2% of amount soil
8. PC0 3%: plant with adding sample C0 3% of amount soil
9. PC1 1%: plant with adding sample C1 1% of amount soil
10. PC1 2%: plant with adding sample C1 2% of amount soil
11. PC1 3%: plant with adding sample C1 3% of amount soil
12. PC2 1%: plant with adding sample C1 1% of amount soil
13. PC2 2%: plant with adding sample C1 2% of amount soil
14. PC2 3%: plant with adding sample C1 3% of amount soil
15. PCL 1%: plant with adding liquid commercial compost 1% of amount soil
16. PCL 2%: plant with adding liquid commercial compost 2% of amount soil
17. PCL 3%: plant with adding liquid commercial compost 3% of amount soil

The implementation of the compost SCG is carried out during the vegetative phase of the plant (21 days). Vegetative phase is the growth phase of the plant from first growing. The juvenile / vegetative phase leads to the formation or growth of leaves, roots, and stems and branching (vegetative organs as source and sink). The mustard seed and soil used are commercial. The type of soil used is pure soil and less on nutrition or without a mixture of fertilizers. Plant samples were obtained from the vegetative phase for nutrient analysis. The samples were oven dried at 65 °C for 48 h to constant weight and ground for nutrient analysis. In all analyses, three repetitions were performed for each sample.

4.3.2.2.Physic and Chemical Analysis

1. Physic analysis

Plant physical analysis was carried out after 3 weeks of seeding. The analysis is to calculate the length of the stem and the width of the leaf. The data is displayed in the form of a graph and figures.

2. SEM and Germination index

Each compost used was observed under SEM (Scanning Electron Microscopy) (Kalab et al., 2008). While for each starter, a Germination Index (GI) analysis was performed (Zucconi et al. 1981). Both types of analysis are to determine the types of microorganisms in the compost as well as to determine their potential for enrichment of compost nutrients

3. Nutrient analysis

In all compost and plant samples, macromolecule and mineral content were analysed. Total Carbon and Nitrogen analysis was done using Macro corder. Meanwhile, mineral content analysis such as Ca, Mg, and K was carried out with AAS 6300, and total phosphorus by UV spectrophotometer. The physical-chemical properties of samples were determined by the standard method (Embrapa, 2009).

4. Biomass

Plants were shaken gently to remove all adhering soil particles, then placed in an oven at 70 C until constant weight to record DW (Dry Weight) as Biomass (Rady et al., 2016). The data is displayed in the form of a graph completed with a quadratic curve.

5. Nutrient uptake

Total uptake of N/P/K was calculated separately by the following formula (Sharma et al., 2012):

$$Nutrient\ \ uptake\ \ (\frac{kg}{ha}) = \frac{\mathrm{N\%\ or\ P\%\ or\ K\%\ x\ dry\ matter\ (\frac{kg}{ha})}}{100}$$

The area to calculate dry matter (kg) in hectares (ha) is by calculating the area of the pot used for hatchery. By adding up the area of the blanket and the bottom of the pot, with the formula below:

Total pot area$= \pi \mathrm{dt} + \pi r^2$

Diameter = 8 cm, and height= 7 cm

4.4.Results and Discussion

4.4.1. Material Properties

Material properties used in this research were soil and SCG compost. The chemical properties of the soil are represented in Table 4.1. The nutrient content of the compost is represented in Table 4.2.

Table 4.1. Chemical properties of soil

Soil properties	Values
Total Carbon (%)	28.88
Total Nitrogen (%)	0.92
pH	6.33
EC (ms/cm)	1.44
CEC (meq/100g)	16.53
Phosphorus (mg/l)	2.77

Data are expressed as mean of three replicates

Table 4.2. Nutrient content of SCG Compost

Nutrient	C0	C1	C2
Total Carbon (%)	17.3	15.28	15.49
Total Nitrogen (%)	2.07	2.02	2.22
C/N ratio	8.33	7.56	6.97
Phosphorus (%)	2.42	5.64	3.29
Potassium (%)	3.73	3.75	4.15
Magnesium (%)	7.83	9.54	8.07
Calcium (%)	12.36	21.18	9.537
pH	7.68	7.53	7.67
EC (ms/cm)	6.27	6.98	6.94
Population of microorganism (log cfu/g)	7.01	6.98	6.94

Data are expressed as mean of three replicates

From the results of the analysis in table 1, soil pH is included in the slightly acidic category (6.33). While the SCG compost showed a slightly alkaline pH (7.53-7.68). The use of SCG compost is expected to increase soil pH so that it becomes normal and is better for seeding mustard plants. Soil pH plays an important role in determining whether nutrient elements are easily absorbed by plants. Nutrients are generally well absorbed by plants at a neutral pH. Nitrogen (N) has an important role for plant growth. Nitrogen can be absorbed by plants from the soil in the form of NH_4^+ and NO_3^-. Total N is a macro element needed by plants in large quantities and makes up 1.5% of the plant weight (Mahler, 2004). N functions

in the formation of protein (Hanafiah 2009). The availability of nitrogen in the soil is included in the medium category (0.92%). Meanwhile, the nitrogen content in compost is high (around 2%).

The phosphorus (P) content in the soil is in the low category. The P content of soil is 2.77 mg / l or equal to 0.027%. While compost SCG is classified into high category up to 2-3%. This is very good, the addition of SCG compost can increase soil phosphorus thus increasing plant nutrition. Next to nitrogen, phosphorus (P) is the second most important macronutrient as an essential plant nutrient (Srinivasan et al., 2012). It is a key nutrient for higher and sustained agricultural productivity (Scervino et al., 2011) and which limits plant growth in many soils. Phosphorus forms an important component of the organic compound adenosine triphosphate (ATP), which is the energy currency that drives all biochemical processes in plants (Brady et al., 2008). It is also an integral component of nucleic acids, coenzymes, nucleotides, phosphoproteins, phospholipids and sugar phosphates as well as intermediates of signal transduction events (Khan et al., 2010; Plaxton et al., 2015; Razaq et al., 2017). It is also involved in an array of processes in plants such as photosynthesis, respiration, nitrogen fixation, flowering, fruiting, and maturation (Brady et al., 2008; Plaxton et al., 2015; Xiang et al., 2012). Despite the important role played by soil P in plants, however, phosphorus deficiency in soil is the most common nutritional stress in many regions of the world, affecting 42% of the cultivated land in the world (Liu et al., 1994). The P deficiency is caused either by low P content in the soils parent materials or by transformations of P.

Potassium (K) in SCG compost is classified as very high. Elemental K used by plants is only a small part. The potassium that is dissolved and the potassium that is exchanged is potassium which is considered available. Herawati (2015) explains that the K ion is classified as an element that is easy to move so that it is easily lost from the soil through washing, because K is not firmly held by the soil colloid surface. The high K in SCG compost is also an advantage over compost when added to slightly acidic soil. The cause of high and low potassium in the soil is influenced by the parent material and also the soil pH. Acidic soil pH will cause an increase in potassium fixation, causing a decrease in the availability of elemental K in the soil.

Cation exchange capacity (CEC) is one of the chemical properties of soil that is closely related to the availability of nutrients for plants and is an indicator of soil fertility. CEC is the capacity of soil to absorb and exchange cations. CEC is influenced by soil content, soil type and organic matter content. Soil CEC describes soil cations such as Ca, Mg, Na, and can be

exchanged and absorbed by plant roots (Herawati 2015). From table 1, the land CEC is in the medium category, 16.53 meq / 100 gr.

4.4.2. SCG compost with PGPF

After analysing the number of microorganisms in SCG compost, it was found that the compost still contained many microorganisms, C0 (log 7.01 cfu / g), C1 (log 6.98 cfu / g), and C2 (log 6.94 cfu / g). The observation was continued by observing each SCG compost under SEM (Scanning Electron Microscopy) to determine which type of microorganism grew the most in the sample. The three samples showed that the majority of microorganisms in SCG compost were fungi (Figure 4.2). The composting process at 30^0 Celsius is a good temperature for mushroom growth. Until the end of composting the mushrooms were still there even though they had decreased a lot. This is different from natural composting, where at the end of composting, usually microorganisms will experience a death phase until the number drops dramatically. This very significant reduction is usually due to natural composting, temperatures can reach 60-70^0 Celsius (thermophilic phase), so that many microorganisms will die in this phase.

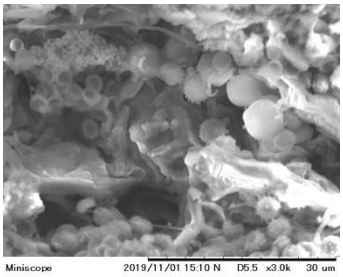


Figure 4.2. SCG compost (C2) observation under SEM, magnification 3000 times

The availability of fungi in the SCG compost is the potential of this compost. Especially because the types of fungi contained in the compost are *Aspergillus* and *Penicillium*. These two types of fungi are PGPF (Plant Growth Promoting Fungi). PGPF are heterogeneous groups of nonpathogenic saprotroph fungi (Hossain, 2020). PGPF exhibit traits beneficial to plants and as such, their capacity to enhance plant growth and development is well founded. PGPF mediates both short- and long-term effects on germination and subsequent plant performance. Improvement in germination, seedling vigor, shoot growth, root growth, photosynthetic efficiency, flowering, and yield are the most common effects decreed by PGPF.

In addition to observations under SEM, a Germination Index (GI) analysis is also carried out on both commercial and fungal starters (Figure 4.3). This is to determine the effect of using a starter that is rich in microorganisms on plant growth. The GI results in the starter

fungi show a higher value (200.4%) when compared to the GI in commercial starters (164.84%), as described in Table 3.

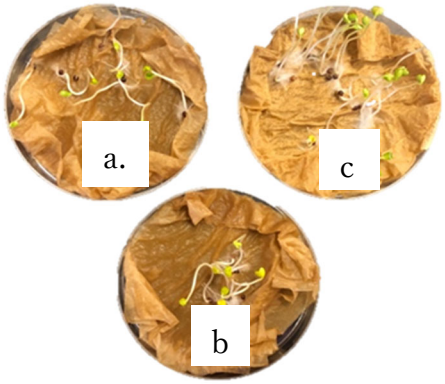


Figure 4.3. (a) Germination Index of plants with no starter; (b) Germination Index of plants with commercial starter; (c) Germination Index of plants with fungi starter.

From Figure 4.3 above, it can be seen that the application of starter fungi to mustard plant seeds gives excellent results when compared to commercial starter applications. More seeds sprouted, and the roots grew longer.

Table 4.3. Germination index data for each starters

Starter	Seed germination (%)	Root Elongation (%)	Germination index (%)
1. Fungi	120	167	200.4
2. *Bacillus*	108	156	164.84

Data are expressed as mean of three replicates

In the previous study, treatment with PGPF, particularly of the genus Aspergillus and Penicillium has been reported to improve seed germination and seedling vigor in different agronomic and horticultural crops. Scarified seeds inoculated with spores from *Aspergillus* had significant increases in germination of Utah milkvetch (*Astragalus utahensis*). The *Aspergillus* treated seeds performed an increase of 30% in seedling cucumber plants (Hossain, 2017). *Penicillium spp* also enhanced leaf chlorophyll content in cucumber and chili (Hossain, 2020)

4.4.3. Physical Analysis

The addition of compost has a very good impact on the growth of mustard plants. After 3 weeks of seeding, the plant stems are taller and the leaves are wider, as shown in Figure 4.4.

This of course is closely related to the macro mineral content in the compost and soil which is absorbed by plant roots. Whereas in the control treatment without compost, plant growth was inhibited due to lack of nutrients, such as Nitrogen, Phosphorus, Potassium, Magnesium, Calcium.

According to Gardner et al (1991), stems is a competitive area within hoard the results of plant assimilation, so that observations of plant height used to determine physical diversity Plants that make a large contribution in forming dry plant matter. Leaves are a carbohydrate factory, where the more the number of leaves on a more and more plants light that can be converted into photosynthate through the process of photosynthesis.

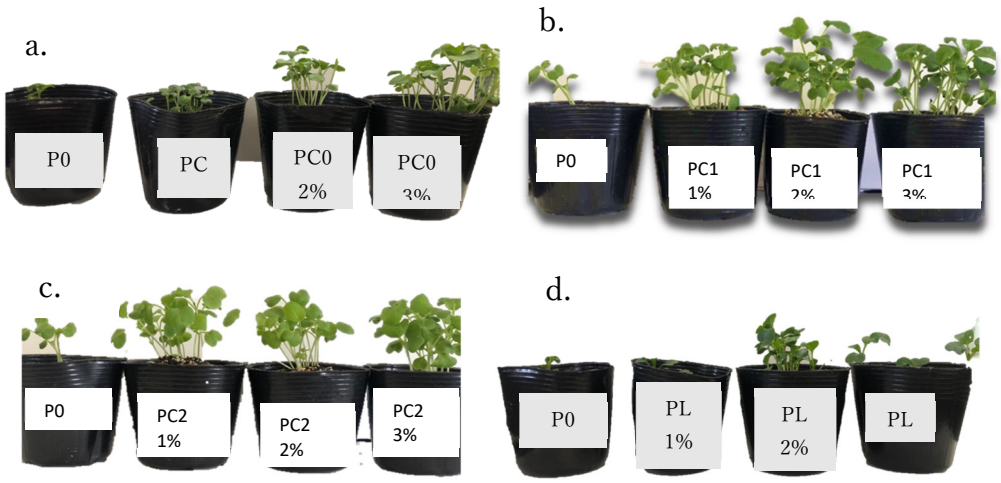


Figure 4.4. (a). Plant growth after implementation of SCG compost control; (b). Plant growth after implementation of SCG compost C1; (c). Plant growth after implementation of SCG compost C2; (d). Plant growth after implementation of commercial compost

After observing the physical plants, the height and width of the leaves were also calculated (Figure 4.4).

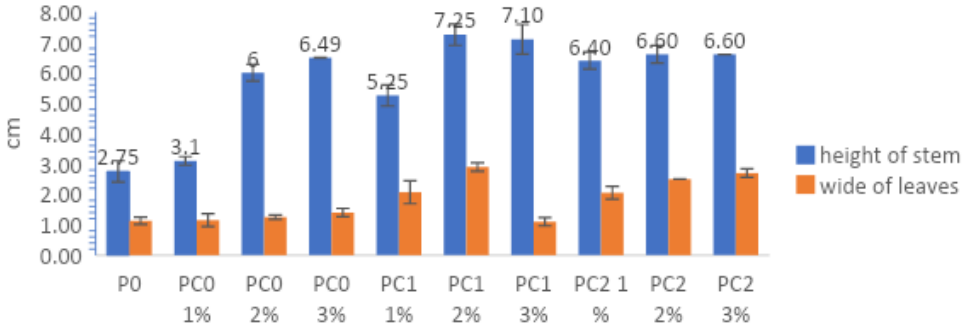


Figure 4.5. Physical of Mustard Plants after 3 weeks seeding

From Figure 4.5 above, it can be seen that the addition of SCG compost is very good for plant growth. When compared with the control, after 21 days of seeding, the length of the stem and leaf area became longer and wider. SCG compost with the addition of commercial starter (C1) at 2 and 3% has a better effect on mustard plants than adding SCG compost with fungi starter (C2). The addition of SCG compost to the seeding mustard plant had better results for stem height and leaf width when compared to no compost added at all (P0). This proves that compost SCG has good nutrition for plant seeding. Primarily are nutrients that plants need for the growth of leaves and stems, such as nitrogen, magnesium, phosphorus, and potassium (macronutrient). According to Rosmarkam (2007), plants that are sufficiently supplied with N can stimulate plant vegetative growth, including increasing plant height, making plants greener because they contain lots of chlorophyll, and are a building block of protein and fat.

Sutiyoso (2003) also mentions that the increase in leaf width is significant because it is influenced by nutrients in organic fertilizers. Leaf width growth is influenced by sufficient N content for plants. In addition to sufficient N nutrients, it is also caused by sufficient Mg levels. Magnesium (Mg) is a nutrient that plays a role in the formation of chlorophyll, activating the phosphorylation process that supports the work of Phosphorus (P) in the energy transfer of ATP (adenosine triphosphate). In addition, at the time of seeding the macronutrient that is no less important is potassium (K). Potassium (K) is the most abundant inorganic cation, and it is important for ensuring optimal plant growth (White and Karley, 2010). K is also very important for cell growth, which is an important process for the function and development of plants (Hepler et al., 2001). As mentioned in Table 2, macronutrient content in SCG compost is very high. Therefore it is better to stimulate plant growth.

4.4.4. Nutrient Analysis

Analysis of plant nutrition after seeding is very important to do. To determine the effect of compost implementation on plant growth, especially during the vegetative period of the plant. The results of nutrient analysis mustard plants are represented in table 4.4.

Table 4.4. Nutrient content in mustard plants after seeding

Samples	C (%)	N (%)	P (%)	K (%)	Ca (%)	Mg (%)
P0	32.11	7.11	7	7.87	71	17.92
PC0 1%	30.68	6.74	3.38	155.75	86.75	12.25
PC0 2%	31.36	6.85	12.28	180	87.75	10
PC0 3%	31.75	6.90	29.9	605	67.5	13
PC1 1%	31.82	7.16	13.1	256.25	86.75	16.25
PC1 2%	32.12	7.32	15.23	222.5	87.75	18
PC1 3%	32.65	7.56	17.19	525	81.25	29.25
PC2 1%	30.82	7.57	19.01	460	92	17.25
PC2 2%	31.77	7.59	13.05	650	98	16
PC2 3%	32.48	7.68	6.38	837.5	83	15.5
PCL 1%	30.55	7.14	14.68	122.5	65.75	8.25
PCL 2%	30.83	7.22	14.76	155	74	8.25
PCL 3%	31.28	7.31	12.71	427.5	58.5	10

Data are expressed as mean of three replicates

From table 4 above, plants without fertilizer application (P0) have higher macronutrient content such as Carbon, Nitrogen, Phosphorus, Potassium, Calcium, Magnesium when compared to plants that have been given SCG compost and commercial compost. From table 4, it can also be seen that the addition of commercial compost has a lower macronutrient than the addition of SCG compost.

Sample P0 has the lowest macronutrient. This is because the nutrients for plant growth are inadequate. The addition of SCG compost increases the nutrients in the plant. The best addition of compost SCG for plants is 3%. This is almost similar to previous research by Chrysargyris et al (2020), on horticultural cabbage. Seed emergence was stimulated in 2.5% of SCG compost. The incorporation of SCG compost impacted the mineral content accumulated in plants with increases in nitrogen, potassium, and phosphorus and decreases in magnesium and calcium.

Soheil et al. (2012) determined the effects of Municipal Waste Compost (MWC) on corn plant responses in pot experiment. They also tested the concentrations of N, P, K and micronutrient elements in the dry matter of the aerial part of the plant. The result showed that

N, P and K content and concentration of micronutrients in plant increased with increase of compost concentration. Amount of the waste compost was significantly increased concentrations of macro and micronutrients in dry matter.

In Table 4 above, it can also be seen that commercial compost has an effect on increasing nutrients in plants when compared to compost plants. However, when compared to plants treated with SCG compost, plant nutrients with commercial compositions were still lower. This proves that, compost SCG has a better effect on mustard plants.

4.4.5. Biomass

Biomass analysis on generally can be used as an illustration the ability of plants to accumulate dry material that can be used as hints of growth traits. Higher the amount of photosynthate means more a lot of dry matter that can be stored (Jumin, 2010). Data biomass of mustard plants can be seen in curve Figure 4.6

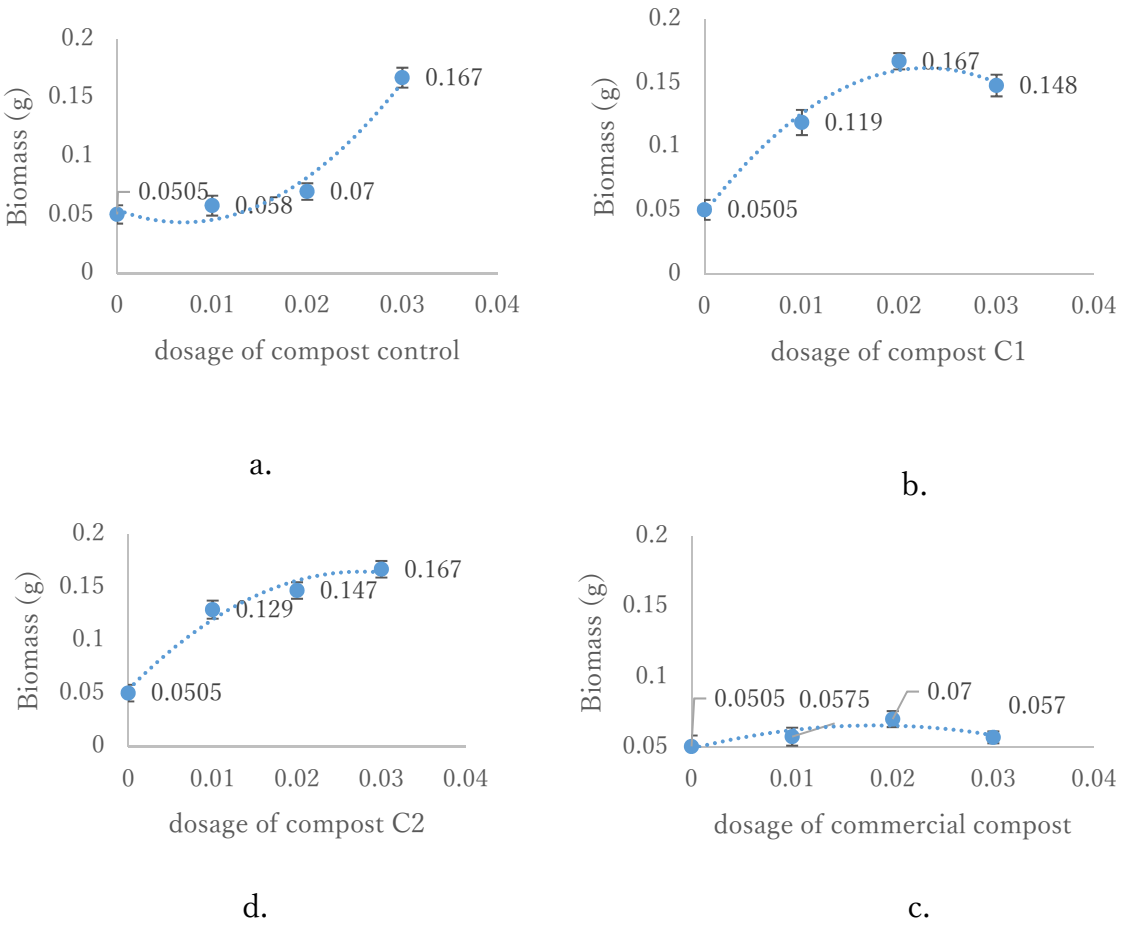


Figure 4.6. Curve of Mustard Plant Biomass in Variation dosage of Compost

From Figure 4.6 above, the addition of control compost yields lower biomass at 1 and 2%. However, at the addition of 3%, biomass increased significantly. This is because, based

on Table 2, the nutrients in the compost control (C0) are lower when compared to C1 and C2. So that to produce high biomass, more compost is needed.

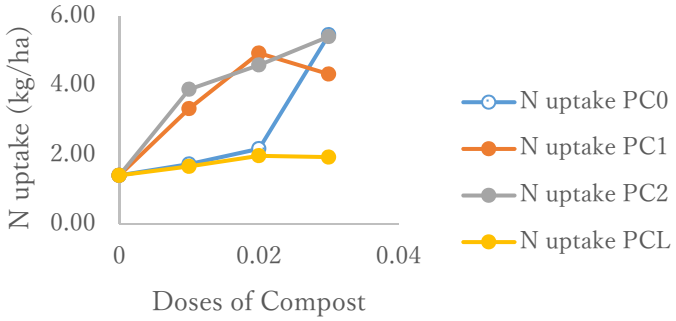
Biomass at the addition of compost C1, optimal at the addition of 2% dose. The addition of 3% compost dosage actually decreased the biomass yield. Slightly different from the addition of C2 compost, optimal compost dose is 3%. Meanwhile, the biomass in the addition of commercial compost shows an even smaller value when compared to the addition of compost control. This proves that the mineral content or nutrients in plants with the addition of SCG compost, both compost control, C1, and C2 are more than the mineral content in plants with the addition of commercial compost. This is also evidenced by the results of nutrient analysis which have been described in Table 4.

According to Harjadi (1984), plant growth is a function of efficiency in producing dry matter plant. Dry weight tightly to do with increased growth and development in absorbing nutrients for growth and development of the vegetative part. If dry weight is low then growth Vegetative plants will be inhibited, because of the nutrients which is absorbed a little so that it affects plant growth.

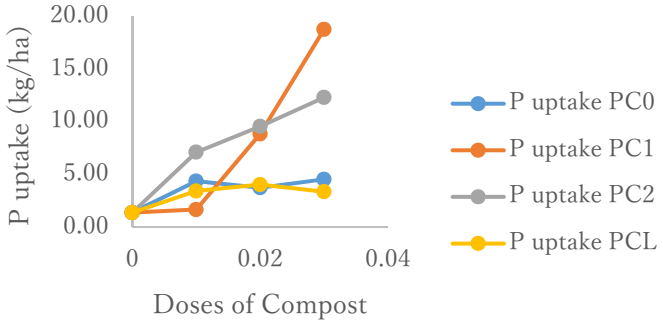
Plant dry weight reflects nutrient status and the amount of nutrients absorbed by plants and the rate of photosynthesis. Nutrients in plants play a role in the process of plant metabolism for producing dry material which it depends on the rate of photosynthesis. When the rates of photosynthesis are different, then the amount of photosynthate produced is also different, likewise with the dry weight of the plant is a reflection of the plant growth rate. Prawiranata et al. (1988) states the dry weight of a plant is the result of deep photosynthate build-up its formation requires nutrients, water, CO_2 and sunlight. This condition is supported by the opinion of Lakit (2004) which states that the dry weight of the plant reflects accumulation of organic compounds is the result of plant synthesis from compounds inorganic originating from water and carbon dioxide thus contributing to weight dry the plant.

4.4.6. Nutrient uptake

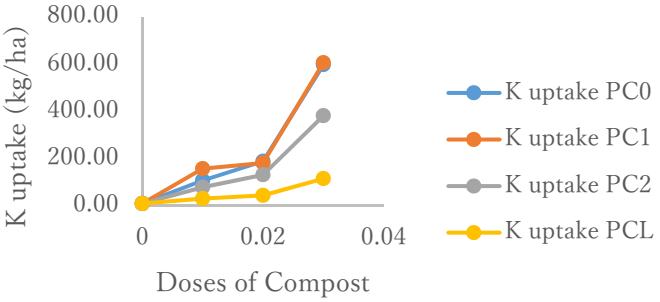
Data on phasic changes related to uptake of nutrients are presented in Figure 4.7. At 21-day growth stage, minimum concentration of nutrients (nitrogen, phosphorus, and potassium) in Mustard plants as well as their uptake was observed P0 and PCL. However, maximum was observed in control plots and increased subsequently with increase doses of compost (PC2). Minimum nitrogen uptake of 1.67 (PCL 1%) and 1.42 kg/ha (P0) and maximum of 5.42 (PC2 3%) and 5.47 kg/ha (PC0 3%). Nitrogen concentration varied between 6 to 7% in the samples (Table 4).



(a.)



(b.)



(c.)

Figure 4.7. Nutrients uptake by Plants

Phosphorus uptake varied between 1.39 (P0) to 19.5 kg/ha (PC1 3%). Following the similar trend of uptake, increase in concentration of phosphorus in mustard plants was a function of increasing doses of compost, it ranged between 3-15 %, whereas in control it was 7 %. Minimum potassium uptake (7.87 kg/ha in control), whereas maximum potassium uptake of 605 kg/ha (PC1 3%).

From the data above, the nutrient uptake by plants added with commercial compost is better than plants that are not added with compost. However, when compared to nutrient uptake

by plants that have been added with compost, both control compost (C0), compost with starter fungi (C2), and compost with commercial starter (C1), and have a much higher nutrition uptake.

The addition of compost to the soil resulted greater biomass and nutrient uptake by Mustard plant, compared to where compost was not added to the soil. The concentration increased with compost addition. Thus, it is likely that the improvements in biomass associated with compost addition were in part due to soil P amelioration likewise with nitrogen (Nguyen et al., 2013; Duong et al., 2012; Hargreaves et al., 2008).

CONCLUSION

Adding compost have positive effect for plant growth especially for length of stem and width of leaves. Plants with the addition of SCG compost (C2) as much as 3% have the best results when compared to the addition of commercial compost (C1) and compost control (C0) in terms of plant physical and nutrients contained therein. Likewise with the biomass produced. The results of the in vitro germination index analysis also proved that the fungi starter implemented in the Mustard plant had the best GI value, namely 200.4%.

REFERENCES

Afriliana A, Endar H, Yishiharu M, Taizo M, Hiroyuki H. 2020. Study on Composting SCG Using Aspergillus sp and Penicillium sp in Aerobic Static Batch Temperature Control. Journal of Chemistry and Environmental Science. In press.

Aly AH, Debbab A, Proksch P. 2011. Fungal endophytes: Unique plant inhabitants with great promises. Applied Microbiology and Biotechnology. 90, 1829-1845.

Armengaud, P., Sulpice, R., Miller, A. J., Stitt, M., Amtmann, A., and Gibon, Y. 2009. Multilevel analysis of primary metabolism provides new insights into the role of potassium nutrition for glycolysis and nitrogen assimilation in Arabidopsis roots. Plant Physiol.150,772–785.doi:10.1104/pp.108.133629

Arora, D.S. and Garg, K.K. 1992. Comparative degradation of lignocellulosic residues by different fungi. Bioresource Technol. 1: 279-280.

Arora, M., Sehgal, V.K., Thapar, V.K., and Wodhwa, M. 1994. Nutritional Improvement of rice straw by higher fungi. Proceedings of National Conference on Fungal Biotechnology, Barakatullah University, Bhopal. p 36.

Bent E. 2006. Induced systemic resistance mediated by plant growth-promoting rhizobacteria (PGPR) and fungi (PGPF). In: Tuzun S, Bent E, editors. Multigenic and Induced Systemic Resistance in Plants. New York: Springer, pp. 225-258.

Bhanawase, D.B., Jadhav, B.R., Rasal, P.H. and Patil, P.L. 1994. 25 years mineralization of nutrients during production of phospho compost. J. Indian Soc. Soil Sci. 42: 145-147.

Brady CN, Weil RR. 2008. The Nature and Properties of Soils, 14th Ed; Pearson Prentice Hall, New Jersey, 975.

Brown S, Gillespie AJR & Lugo AE. 1984. Biomass estimation methods for tropical forests with applications to forest inventory data. For. Sci. 35(4): 881-902. Choirudin & Purwanto

Buckerfield J, Webster K. 1998. Compost as mulch for managing young vines. *The Australian Grapegrowers & Winemaker*, pp. 75–78.

Campbell, L.; Rempel, C.B.; Wanasundara, J.P. 2015. Canola/Rapeseed Protein: Future Opportunities and Directions—Workshop Proceedings of IRC; Multidisciplinary Digital Publishing Institute: Basel, Switzerland

Chrysargyris, A., Antoniou, O., Xylia, P. *et al.* The use of spent coffee grounds in growing media for the production of *Brassica* seedlings in nurseries. *Environ Sci Pollut Res* (2020). https://doi.org/10.1007/s11356-020-07944-9

Collett, M.G.; Stegelmeier, B.L.; Tapper, B.A. 2014. Could nitrile derivatives of turnip (Brassica rapa) glucosinolates be hepato-or cholangiotoxic in cattle? J. Agric. Food Chem, 62, 7370–7375. [CrossRef] [PubMed]

Coskun, D., Britto, D. T., and Kronzucker, H. J. 2016. The nitrogen–potassium intersection: membranes, metabolism, and mechanism. Plant Cell Environ. 10, 2029–2041.doi:10.1111/pce.12671

Deubel, Annette & Merbach, Wolfgang. 2005. Influence of Microorganisms on Phosphorus Bioavailability in Soils. 10.1007/3-540-26609-7_9.

Du, Q., Zhao, X.H., Jiang, C.J., Wang, X.G., Han, Y., Wang, J., et al. 2017. Effect of potassium deficiency on root growth and nutrient uptake in maize (Zeamays L.). Agric. Sci.8,1263–1277.doi:10.4236/as.2017.811091

Duke, J.A. 2002. Handbook of Medicinal Herbs, 2nd ed.; CRC Press: Melbourne, Australia.

Duong, T. T., Penfold, C. & Marschner, P. 2012. Amending soils of different texture with six compost types: impact on soil nutrient availability, plant growth and nutrient uptake. Plant Soil 354, 197–209.

Duputel M, Devau N, Brossard M, Jaillard B, Hinsinger P, Gérard F. 2013. Citrate adsorption can decrease soluble phosphate concentration in soils: Results of theoretical modelling. Appl Geochem, 35:120-131.

Embrapa. 2009. Manual de análises químicas de solo, plantas e fertilizantes. Brasília, 627 p.

Gaind, S., Pandey, A.K. and Lata. 2006. Microbial Biomass, PNutrition, and Enzymatic Activities of Wheat Soil in Response to Phosphorus Enriched Organic and Inorganic Manures. J. Environ. Sci. Health Part B. 41:177–187.

Gardner, F.P., R.B. Pearce dan R.L. Mitchell. 1991. Physiology of Crop Plants (Fisiologi Tanaman Budidaya, alih bahasa H. Susilo). UI Press. Jakarta. 428h.

Gaur, A.C. 1982. A Manual of Rural Composting, FAO-UNDP Regional project RAS/75/004. Field document No. 15. FAO, UN, Roam, Italy. p102.

Hanafiah K. 2009. Dasar-dasar Ilmu Tanah. Jakarta (ID): Raja Grafindo Perkasa.

Hargreaves, J. C., Adl, M. S. & Warman, P. R. 2008. A review of the use of composted municipal solid waste in agriculture. Ag. Ecosys. Environ. 123, 1–14, https://doi.org/10.1016/j.agee.2007.07.004.

Haseena, V.M. Nishad, M. Balasundaran. 2016. A consortium of thermophilic microorganisms for aerobic composting. IOSR Journal of Environmental Science, Toxicology and Food Technology, 10 (1), 49-56.

Herawati MS. 2015. Kajian Status kesuburan Tanah di Lahan Kakao Kampung Klain Distrik Mayamuk Kabupaten Sorong. Jurnal Agroforestri. Edisi X: 201-208

Hossain M.M., Sultana F., Islam S. 2017. Plant Growth-Promoting Fungi (PGPF): Phytostimulation and Induced Systemic Resistance. In: Singh D., Singh H., Prabha R. (eds) Plant-Microbe Interactions in Agro-Ecological Perspectives. Springer, Singapore. https://doi.org/10.1007/978-981-10-6593-4_6

Hossain MM, Sultana F, Kubota M, Koyama H, Hyakumachi M. 2007. The plant growth-promoting fungus Penicillium simplicissimum GP17-2 induces resistance in Arabidopsis thaliana by activation of multiple defense signals. Plant & Cell Physiology. 48(12),1724-1736.

Hossain, Md & Sultana, Farjana. 2020. Application and Mechanisms of Plant Growth Promoting Fungi (PGPF) for Phytostimulation. 10.5772/intechopen.92338.

Hu, W., Jiang, N., Yang, J., Meng, Y., Wang, Y., Chen, B., et al. 2016a. Potassium (K) supply affects K accumulation and photosynthetic physiology in two cotton (*Gossypiumhirsutum L.*) cultivars with different K sensitivities. Field Crop. Res. 196,51–63.doi:10.1016/j.fcr.2016.06.005

Hu, W., Zhao, W., Yang, J., Oosterhuis, D. M., Loka, D. A., and Zhou, Z. 2016b.

Hyakumachi M, Kubota M. 2004. Fungi as plant growth promoter and disease suppressor. In: Arora DK, editor. Mycology Series. Vol. 21. Fungal Biotechnology in Agricultural, Food, and Environmental Applications. New York: Marcel Dekker, pp. 101-110

Hyakumachi M. 1994. Plant-growth promoting fungi from turf grass rhizosphere with potential for disease suppression. Soil Microorganisms. 44, 53-68.

Indonesian Ministry of Agriculture. 2020. Mustard plant cultivation. http://cybex.pertanian.go.id/mobile/artikel/91829/Budidaya-Tanaman-Sawi/

Inonu I, Khodijah NS, Supriadi A. 2014. Budidaya Pakchoy (Brassica rapa L.) di lahan tailing pasir bekas penambangan timah dengan ameliorant pupuk organic dan pupuk NPK. Lahan suboptimal 3(1): 76-82

Ivanova, D. Bojinova, K. Nedialkova. 2006. Rock phosphate solubilization by soil bacteria. Journal of the University of Chemical Technology and Metallurgy. 41. 297-302.

Jin, H. C., Zhang, L. S., Li, B. Z., Han, M. Y., and Liu, X. G. 2007. Effect of potassium on the leaf nutrition and quality of Red Fuji apple. Acta Agric. Bor Occid.Sin.16,100–104.doi:10.3969/j.issn.1004-1389.2007.03.026

Jumin, H.B. 2010. Based on Agronomi. Revision edition. Rajawali Pers, Jakarta.

Kaewchai S, Soytong K, Hyde KD. 2009. Mycofungicides and fungal biofertilizers. Fungal Diversity. 38: 25-50

Kaiser, W. M. 1982. Correlation between changes in photosynthetic activity and changes in total protoplast volume in leaf tissue from hygro-, meso and xerophytes under osmotic stress. Planta 154, 538–545. doi: 10.1007/ BF00402997

Kalab M. 2008. Conventional Scanning Electron Microscopy of Bacteria. Food Microstructure 3(1), 95-111.

Khan MS, Ahmad E, Zaidi A, Oves M. 2013. Functional aspect of phosphate-solubilizing bacteria: Importance in crop production. In: Bacteria in Agrobiology: Crop Productivity. Springer, Berlin, 237-263.

Khan QU, Khan MJ, Rehman S, Ullah S. 2010. Comparison of different models for phosphate adsorption in saly inherent soil series of Dera Ismail Khan. Soil and Environment, 29(1):11-14.

Kim, K. Y., D. Jordan D. and G. A. McDonald. 1997. Solubilization of hydroxyapatite by Enterobacter agglomerans and cloned Escherichia coli in culture medium, Biol. Fert. Soils 24:347-352.

Liu JZ, Li ZS, Li JY. 1994. Utilization of plant potentialities to enhance the bio efficiency of phosphorus in soil. Eco-agriculture Research, 2, 16-23.

Liu, H.-J & Chen, Y.-W & Sun, G.-F & Chen, L.-G & Zheng, J.-C. 2017. Effects of different organic-inorganic fertilizer combination ratios on rice yield and nutrient loss with surface runoff. Chinese Journal of Ecology. 36. 405-412. 10.13292/j.1000-4890.201702.003.

Lu, J.W., Chen, F., Wan, Y.F., Liu, D.B., Yu, C.B., Wang, Y.Q., et al. 2001.Effect of application of potassium on the yield and quality of Navel Orange. J. Fruit Sci. 18, 272–275.

Mahler R. 2004. Nutrients Plants Require for Growth. Department of Plant, Soil, and Entomological Sciences. The University of Idaho, Moscow.

Marschner, H. 2012. Marschner's Mineral Nutrition of Higher Plants. Cambridge, MA: Academic press.

Nesi, N.; Delourme, R.; Brégeon, M.; Falentin, C.; Renard, M. 2008. Genetic and molecular approaches to improve nutritional value of Brassica napus L. seed. Comptes Rendus Biol. 331, 763–771. [CrossRef] [PubMed]

Nguyen, T. T., Fuentes, S. & Marschner, P. 2013. Effect of incorporated or mulched compost on leaf nutrient concentrations and performance of Vitis vinifera cv. Merlot. J. Soil Sci. Plant Nut. 13, 485–497.

Ogawa H & Kira T. 1977. Methods of estimating forest biomass In Primary productivity of japanese forests: productivity of terrestrial communities. Shidei T & Kira T (eds.), Japanese Committee for the International Biological Program, University of Tokyo Press, Japan.

Olibone D, Rosolem CA. 2010. Phosphate fertilization and phosphorus forms in an Oxisol under no-till. Scientia Agricola, 67:465-471.

Oosterhuis, D., Loka, D., Kawakami, E., and Pettigrew, W. 2014. The physiology of potassium in crop production. Adv. Agron. 126, 203–234. doi:10.1016/B9780-12-800132-5.00003-1

Pan, Genxing & Smith, Pete & Pan, Gengxing. 2009. The role of soil organic matter in maintaining the productivity and yield stability of cereals in China. Agriculture, Ecosystems & Environment. 129. 344-348. 10.1016/j.agee.2008.10.008.

Plaxton W, Lambers H. 2015. Phosphorus metabolism in plants. Annual Plant Reviews, 48, 1-480.

Rady, M.M., Semida, W.M., Hemida, K.A. et al. 2016. The effect of compost on growth and yield of Phaseolus vulgaris plants grown under saline soil. Int J Recycl Org Waste Agricult 5, 311–321. https://doi.org/10.1007/s40093-016-0141-7

Rasal, P.H., Patil, P.L., Shingte, V.V., and Kalbhor, H.B. 1990. A role of Azotobacter in enrichment of compost. Proceedings of VIII Southern Regional Conference on Microbial Inoculants, Pune. p 47.

Raviv, Michael. 2005. Production of High- quality Composts for Horticultural Purposes: A Mini-review. Hort Technology. 15. 10.21273/HORTTECH.15.1.0052.

Raymer, P.L. 2002. Canola: An emerging oilseed crop. Trends New Crops New Uses, 1, 122–126.

Razaq M, Zhang P, Shen Hl, Salahuddin. 2017. Influence of nitrogen and phosphorus on the growth and root morphology of Acer mono. PLoS One, 12, 1–13.

Relationship between Potassium Fertilization and Nitrogen metabolism in the leaf subtending the cotton (Gossypium hirsutum L.) boll during the boll development stage. Plant Physiol. Biochem. 101,113–123. doi:10.1016/j.plaphy. 2016.01.019

Ronga, Domenico & Pane, Catello & Zaccardelli, Massimo & Pecchioni, Nicola. 2015. Use of Spent Coffee Ground Compost in Peat-Based Growing Media for the Production of Basil and Tomato Potting Plants. Communications in Soil Science and Plant Analysis. 47. 10.1080/00103624.2015.1122803.

Ruan, J., Wu, X., Ye, Y., and Hardter, R. 1998. Effect of potassium, magnesium andsulphurappliedindifferentformsoffertilisersonfreeaminoacidcontent in leaves of tea (Camellia sinensis L). J. Sci. Food Agric. 76, 389–396. doi: 10.1002/(SICI)1097-0010(199803)76:3<389::AID-JSFA963<3.0.CO;2-X

Rufty, T.W., Jackson, W.A., and Raper, C.D. 1981. Nitrate reduction in roots as affected by the presence of potassium and by flux of nitrate through the roots. Plant Physiol.68,605–609.doi:10.2307/4266953

Ruiz, J., and Romero, L. 2002. Relationship between potassium fertilisation and nitrate assimilation in leaves and fruits of cucumber (Cucumis sativus) plants. Ann.Appl.Biol.140,241–245.doi:10.1111/j.1744-7348.2002.tb00177.x

Scervino JM, Papinutti VL, Godoy MS, Rodriguez JM, Monica ID, Recchi M, et al. 2011. Medium pH, carbon and nitrogen concentrations modulate the phosphate solubilization efficiency of Penicillium purpurogenum through organic acid production. J Appl Microb, 110:1215- 1223.

Shen J, Yuan L, Zhang J, Li H, Bai Z, Chen X, Zhang W, Zhang F. 2011. Phosphorus dynamics: From soil to plant. Plant Physio, 156(3):997-1005.

Shinde, D.B., Jadhav, S.B. and Navale, A.M. 1990b. Recent trends in recycling of sugarcane trash, Deccan Sugarcane Technology Association, part I. pp.249-254.

Soheil R, Hossien M H, Gholamreza S, Leila H, Mozhdeh J and Hassan E. 2012. Effects of Composted municipal waste and its Leachate on Some Soil Chemical Properties and Corn Plant Responses. Int. Journal of Agriculture: Research and Review. Vol., 2 (6), 801-814.

Spragg, J. 2016. Australian Feed Grain Supply and Demand Report 2016; JCS Solutions Pty Ltd.: North Victoria, Australia, pp. 1–42.

Srinivasan R, Yandigeri MS, Kashyap S, Alagawadi AR. 2012. Effect of salt on survival and P-solubilization potential of phosphate solubilizing microorganisms from salt affected soils. Saudi J. Biol. Sci, 19: 427–434

Talashilkar, S.C. 1985. Effect of microbial culture (Azotobacter chroococcum) on humification and enrichment of mechanized compost. Indian J. Agric. Chem. 22: 193-195.

Tisdale SL, Nelson WL, Beaton JD. Soil and fertilizer phosphorus. 1985. In: Soil Fertility and Fertilizers. Macmillan Publishing Company, New York, 189-248.

Tränkner, M., Tavakol, E., and Jákli, B. 2018. Functioning of potassium and magnesium in photosynthesis, photosynthate translocation and photoprotection. Physiol. Plant. 163, 414–431. doi: 10.1111/ppl. 12747

Vimala, P. & Mohd. Noor, Mohamad roff & Shokri, O. & Lim, A.H. 2010. Effect of organic fertilizer on the yield and nutrient content of leaf-mustard (Brassica juncea) organically grown under shelter. J. Trop. Agric. and Fd. Sc, 38, 1-8.

Vujanovic V, Goh YK. 2012. qPCR quantification of Sphaerodes mycoparasitica biotrophic mycoparasite interaction with Fusarium graminearum: in vitro and in planta assays. Archives of Microbiology, 194(8):707-717.

Wahyudi. 2010. Practical Farming Guidelines Vegetables. Agromedia Pustaka. Jakarta.

Wang, Y.Z., Zhang, H.P., Huang, X.S., Wang, J.Z., Cheng, R., Chen, G.D., et al. 2017.Effect of potassium supply on plant potassium distribution and growth and leaf photosynthetic capacity of Pyrus pyrifolia. J. Nanjing Agric. Univ. 40, 60–67.doi:10.7685/jnau.201603054

Watson, R.R., Preedy, V.R. 2010. Bioactive Foods and Extracts: Cancer Treatment and Prevention; Taylor & Francis: Queens Road, Australia.

White,P.J., and Karley,A.J. 2010. Potassium Cell Biology of Metals and Nutrients. Berlin: Springer, 199–224.

Xiang DB, Yong TW, Yang WY, Gong YWZ, Cui L, Lei T. 2012. Effect of phosphorus and potassium nutrition on growth and yield of soybean in relay strip intercropping system. Scientific Research and Essays, 7(3), 342-351.

Xu G, Fan X, Miller AJ. 2012. Plant nitrogen assimilation and use efficiency. Annu Rev Plant Biol 63:153–18.

Whittaker RH & Marks PL. 1975. Methods of assessing terrestrial productivity. Dalam Lieth H & Whittaker RH.(edisi), Primary productivity of the biosphere. Springer-Verlag, New York. 141

Zayed, G. and Abdel-Motaal, H. 2005. Bioactive composts from rice straw enriched with rock phosphate and their effect on phosphorus nutrition and microbial community in rhizosphere of cowpea. Bioresource Technol. 96: 929.

CHAPTER 5

GENERAL CONCLUSION

Chapter 5

Overall conclusion and future directives

In this study for each chapter give brief conclusions, which include comments and summary. The overall concluding remarks of this dissertation, the unsolved analysis for this investigation and the future direction of concerned issues are highlighted in this chapter.

5.1 OVERALL CONCLUSION

Technology of refermentation inside of automatic reactor using kefir starter can increase quality of coffee bean. Kefir microbial community, which is generally composed of different lactic acid bacteria (LAB), acetic acid bacteria (AAB), and yeast species (Farnworth, 2005). Presence some metabolic products by kefir such as organic acids (lactic acid, acetic acid, butyric acid, propionic acid, and citric acid). This organic acid plays a role in reducing the pH after the coffee refermentation process. The fermentation temperature has a significant effect on the pH of the coffee beans. Fermentation using a temperature of 37ºC has the lowest pH value compared to seeds coffee is fermented using temperatures of 27 and 47ºC. The total lactic acid bacteria at 37ºC fermentation with time 12 and 18 were not significantly different, namely 7.59 and 7.76 log cfu / g. This value is higher than in temperature 27 and 47ºC.

Refermented beans for 12 hours produce the best score of cup test, 80.5. This value is up almost 5 points from the control or sample of unfermented Robusta coffee 75.87. This indicates that refermented Robusta beans have succeeded in entering the specialty coffee category. To be classified as specialty coffee, a coffee needs to obtain a quality score of 80 or higher on a 100-point scale from the coffee-tasting process (Specialty Coffee Association of America [SCAA], 2016). The characteristics assessed on the cup test are aroma, flavour, after taste, acid / salt, bitter / sweet and balance.

Beside cup test analysis, we also conduct volatile compound analysis. Volatile compounds have an important role in quality coffee (Sunarharum, 2016). Based on the identification of volatile compounds in unfermented Robusta coffee (control) and refermented using kefir, shows during refermentation an increase in the number of volatile compounds that can be identified. 17 compound of control Robusta coffee beans were identified, while the fermentation treatment with kefir starter at 37ºC shows that it increases with the time of fermentation. Fermentation during 6, 12 and 18 hours were identified 21, 22 and 23 volatile

compounds, respectively. Some compounds group including of acid, alcohol, aldehyde and acetate groups were contributed to acidy, fruty, nutty and caramelly aroma.

Coffee grounds from the results of this refermentation technique, with the minimum content of caffeine and polyphenols, have potential if implemented for plant growth. In this study, we continue our study to utilize SCG (Spent Coffee Ground) from refermented bean. We studied composting of SCG by the addition of some fungi as starter culture with temperature control, such as *Aspergillus sp* and *Penicillium sp.* Combination of this fungi with temperature control in composting SCG can improve quality compost produced, with the physical characteristics of compost black and crumb, and normal pH. While the chemical characteristics of compost produced is a C/N ratio below 10 with a far difference from the control. Compost is also richer in minerals, such as phosphorus, potassium, calcium, and magnesium, as well as rich in humic acid as shown from the results of the FTIR analysis. Addition of a combination of fungi starter such as *Aspergillus sp*, and *Penicillium sp* can compete with commercial activators. This is also evidenced from the results of the phytotoxicity analysis, where the Germination Index of the compost sample with the addition of fungi activator (C2) is 191.86% greater than the commercial activator (C1) 183.88%.

SCG compost then apply to plant growth for pot treatment. SCG compost which has been enriched with inoculant starter for composting can stimulate plant growth more when compared to commercial compost both organic and inorganic compost. This is evidenced by the longer the plant stem and the wider the leaves. In addition, the results of plant macronutrient content analysis also showed that the addition of compost during seeding can improve plant nutrients such as phosphorus, nitrogen, potassium, and other macro minerals so that it had a good impact on plant growth. Plants with the addition of SCG compost (C2) as much as 3% have the best results when compared to the addition of commercial compost (C1) and compost control (C0) in terms of plant physical and nutrients contained therein. Likewise, with the biomass produced. The results of the in vitro germination index analysis also proved that the fungi starter implemented in the Mustard plant had the best GI value, namely 200.4%.

5.2 FUTURE DIRECTIVES

To understand more deeply about the refermentation technology using the kefir starter. In the future, it is necessary to identify the types of microorganisms in kefir, then study each of the resulting metabolisms and what enzymes play a role. In addition, the chemical properties change in the coffee beans after the refermentation process. This is of course because the

chemical properties also have an influence on the quality of coffee and its taste. One example is caffeine which affects the bitter taste of coffee beans.

Likewise, with SCG compost, it is necessary to study how much the compounds in it have a toxic effect on plants. Such as caffeine, tannins, and polyphenols. For polyphenols, it is also necessary to research the types of polyphenols. Because not all polyphenols have a negative effect on plants. For starter fungi, it is also necessary to investigate the fungal species using PCR analysis, as well as their metabolism during the composting process which can degrade organic material from SCG and others material. In addition, to maximize the quality of compost, in the future this compost has the potential to be made nano compost so that nutrition release can be slowed down and have good effects not only in the short term, but also in the long term as well for plant growth.

While in pot treatment, in the future, to really know the effect of PGPF (Plant Growth Promoting Fungi) on plants, it is necessary to analyze the types of microorganisms or fungi in plant roots using SEM (Scanning Electron Microscopy). In addition, the analysis of pot treatment is further extended not only during the vegetative period but also during the generative period of the plant. This is to determine the long-term effect of compost on plants. It is also good to analyze the effects of compost on the soil. Therefore, the benefits of compost can be known for both plants and soil.

Appendix A

List of Publications:

Main Publications

1. Studies on Composting of Spent Coffee Grounds by *Aspergillus* sp and *Penicillium* sp in Aerobic Static Batch temperature control, Asmak Afriliana, Endar Hidayat, Yoshiharu Mitoma, Taizo Masuda, Hiroyuki Harada, ***Journal of Agriculture Chemistry and Environment,*** 10, 91-112, 2021.
2. Volatile compounds change in unfermented robusta coffee by refermentation using commercial kefir, Asmak Afriliana, Endar Hidayat, Yoshiharu Mitoma, Taizo Masuda, Hiroyuki Harada, ***Nutrition and Food Science International Journal*****,** 8, 4, 1-5, 2019.
3. Phosphorus Adsorption and Nitric Acid Reduction by Ferrous Sulfate-Treated Foamed Waste Glass, Asmak Afriliana, Hiroyuki, H., Yumi, K., Hanami, N., Yoshiharu, M. and Miyamoto, T. ***Journal of Materials Science and Chemical Engineering***, **6**, 21-30, 2018.

Sub Publications

1. Effects of Co-Existing Ions on the Phosphorus Potassium Ratio of the Precipitate Formed in the Potassium Phosphate Crystallization Process, Harada, H. , Katayama, Y. , Afriliana, A. , Inoue, M. , Teranaka, R. and Mitoma, Y. J***ournal of Environmental Protection***, 8, 1424-1434, 2017.
2. Land Suitability of Coffee in Tokunosima Island Japan, Endar Hidayat, Asmak Afriliana, Gusmini, Hiroyuki Harada, ***Journal of Applied Agricultural Science and Technology*** 4(2),1 – 9, 2020.

Appendix B

Contribution to scientific forums:

1. Asmak Afriliana, Endar Hidayat, Yoshiharu Mitoma, Taizo Masuda, Hiroyuki Harada, Fermented Technology of Robusta Coffee Beans (Canephora Coffee) with Kefir Milk to Produce Specialty Coffee. *4th International Conferences on Food, Agriculture, and Natural Resources*, Yogyakarta, Indonesia, 12 - 14 September 2018.

2. Asmak Afriliana, Endar Hidayat, Yoshiharu Mitoma, Taizo Masuda, Hiroyuki Harada, Evaluation of Potency Spent Coffee Grounds for Make Black Compost. *The International Conference on Agriculture and Life Sciences*, Jember, Indonesia, 31st July- 2nd August 2019.

3. Asmak Afriliana, Yoshiharu Mitoma, Taizo Masuda, Hiroyuki Harada, Technology Refermentation to Produce Specialty Coffee. *Advance Innovation Global Competition*, Nanyang Technological University, Singapore, 15 – 17 November 2019.

Appendix C

Curriculum Vitae of

Asmak Afriliana

Personal Information:	Contact Information:
Date of birth: 1 April 1988, Indonesia Place of birth: Situbondo, Indonesia Gender: Female Marital status: Married	Address: Dept. of Environmental Engineering, Prefectural University of Hiroshima, Japan Email: Asmak.ftp@unej.ac.id Phone: +818085570370 +6285258054505

Education:

2021

Ph.D. in Environmental Engineering (due to complete in March 2021)

Dept. of Environmental Engineering, Prefectural University of Hiroshima, JAPAN

2013

M.Agr. In Food Science

Dept. of Agricultural Product Technology, Brawijaya University, INDONESIA

2010

B.Sc. in Food Science

Dept. of Agricultural Product Technology, Jember University, INDONESIA

2006

High School certificate

Public High School, SMAN 1 Glagah Banyuwangi, INDONESIA

Record of Employment:

2010-2011
Coffee Entrepreneur
Jember, Indonesia

2014~
Lecturer
University of Jember
Jember, Indonesia

2016~
Owner of CV Asmak Kopi
Jember, Indonesia