EFFECTS OF COOKING PROCESSES ON THE TOTAL PHENOLIC CONTENTS AND RADICAL SCAVENGING ACTIVITIES OF INDONESIA AND TAIWAN MUNG BEAN

PRACTICAL TRAINING REPORT

This practical training report is submitted for the partial requirement for Bachelor Degree

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SEMARANG

2018

EFFECTS OF COOKING PROCESSES ON THE TOTAL PHENOLIC CONTENTS AND RADICAL SCAVENGING ACTIVITIES OF INDONESIA AND TAIWAN MUNG BEAN

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This Practical training report has been approved and supported by examiner in Practical Training Exam on June, 4th, 2018

Semarang, June, 4th, 2018

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PREFACE

Thanks to Jesus Christ because only by his blessing and grace, the author have a opportunity to do the practical training and finish the report. The practical training was composed to qualify the graduation course in Soegijapranata Catholic University and this report is the complete accountability from the practical training which was done in Fu Jen Catholic University, New Taipei City, Taiwan that take place from 4th of January until 4th of March, 2018. During the practical training, the author did the research entitled : "Effect Of Cooking Processes On the Total Phenolic Contents and Radical Scavenging Activities Of Indonesia and Taiwan Mung Bean". This report also written and arranged as a requirement to acquire Bachelor Degree of Food Technology in Soegijapranata Catholic University, Semarang, Indonesia. The author would not be able to finish these tasks alone, and only by huge support and guidance that had given by people around, these report could be finished. Special thanks for:

1. Jesus Christ that always blessed, saved and guided the author in every step of practical training in Taiwan.
2. Dr. R. Probo Y. Nugrahedi, STP, MSc. as dean of faculty of agriculture technology Soegijapranata Catholic University for giving the author opportunity to join the internship program.
3. Dr. Jung-Feng Hsieh, Ph. D as author's advisor in Fu Jen Catholic University for giving advices and supporting the author all the time during the practical training.
4. Dr. A. Rika Pratiwi, MSi. as author's advisor in Soegijapranata Catholic University for taking care of the author during the practical training and making the report.
5. Emma, Matt, Bling-Bling, Kevin, Brian, Alice, Ariel, and Sunny who always help the author to do the research and make the report.
6. My family, Dad, Mom, and sister who always support and cheer for the author everyday.
7. Christopher, Angel, Fanny, Eileen, Ken, Daniel, Kevin, Donna, Dave, Giant and all my friends that cannot be mentioned one by one who always support and accompany the author during the practical training until finishing the report.

The author realizes that this report is still far from perfect and there are still many short comings due to the limitation of the author. However, the author hopes that this report can be an inspiration and provide useful information for all the reader.

Semarang, June, 4th, 2018

Author,

Evan Fajar Alim

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1. INTRODUCTION

1.1. Background

Nowadays, food become the most important things and the main needs of human to living in the world. People nowadays things that the most important things in food is delicious and cheap. Even though nutrition and safety in food is the real most important in food appearance. This mindset of people nowadays is a problem because what we eat is affected us. Therefore, we should change the mindset people which only have mindset that are delicious and cheap to have a mindset that are delicious, cheap, nutritious, and safety. Some food industries already developing and applying some modern food manufacturing so, that can enhanced food safety. Moreover modern food manufacturing can maintain and preserved important nutrition like vitamins, minerals, and other sensitive food component. Based on exciting fact, there were many sensitive food component which have a high nutrition value. Many aspect which should be a concern in food industries so that can maintain food quality and enhanced food safety so that can making people not worried about food quality and food safety and also fulfill demand of customer nowadays. that aspect is like food microbiogy, food quality management, food quality assurance, food chemical, and food waste management.

For that reason, Department of Food Technology, Soegijapranata Catholic University (SCU) sets up a training program to let the students improve their skills and knowledges. In this program, the student is given an opportunity to take part in food industry (Practical working) or in-house training (doing research in the selected university). Student who choose to take part in a food industry will know the working system in food industry and real practices and gains a big view about how actual food-related research is executed in the industry. This experience will be helpful once the student graduates and goes into the working world. So, graduated student not surprised if after graduate work in food industry because already know the working system in food industry. While student who chooses in-house training have to go to the selected university to be their facilitator of research. Food Science Department, Fu Jen Catholic University (FJU), Taiwan, is the selected university for in-house training which is sophisticated in applied biotechnology and microbiology aspect. Through this program, the student have given an opportunity

to conduct their research in abroad, having a experience to introduce the cultural diversity in abroad exactly about food culture in abroad. This program can be held because there is a student exchange mutual agreement between SCU (Soegijapranata Catholic University) and FJU (Fu Jen Catholic University).

The title of the research was "Effect of Cooking Processes on the Total Phenolics Contents and Radical Scavenging Activities of Indonesia and Taiwan Mung Bean". The advisor of this research was Prof. Jung-Feng Hsieh, as the Assistant Professor of Food Science Department, Fu Jen Catholic University, Taiwan. The mentor of this research was Emma as the student of Master's Degree program of Food Science Department, Fu Jen Catholic University, Taiwan.

1.2. Purpose of Practical Training

a. To know how to analyze radical scavenging activity using DPPH method.
b. To know how to analyze total phenolic content.
c. To know the effect of cooking processes on the total phenolic contents and radical scavenging activity.
d. To know the different between Indonesia and Taiwan mung bean on total phenolic contents and radical scavenging activity.
e. To understand how to prepare the sample before being analyze.

1.3. Time and Place of Practical Training

The practical training was conducted in Food Science Department, Fu Jen Catholic University, Xinzhuang District, New Taipei City, Taiwan. This activity took place between January 4th until March 4th 2018.

2. INSTITUTION PROFILE

2.1. Fu Jen Catholic University

Fu Jen Catholic University (FJU) is a private university located in New Taipei City, Taiwan. Fu Jen was originally established in Beijing in 1925 by the Benedictines of St. Vincent Archabbey in Latrobe as a single college named Fu Jen Academy. In 1929, Fu Jen was officially recognized as a university by the Ministry of Education. In 1959, the Chinese Regional Bishops' Conference, the Society of Jesus, and the Society of the Divine Word collaborated on the reestablishment of the university in Taiwan. In 1960, the Ministry of Education granted permission to restore Fu Jen in Taiwan, and in 1961 the Graduate Institute of Philosophy admitted students. In 1963, the University was granted a share of the successful candidates of the University Entrance Examination and received the first freshmen of the College of Liberal Arts, Science and Engineering, and Law.

Fu Jen has a history of more than 90 years. Five decades after its reestablishment in Taiwan, more than 190,000 students have graduated from Fu Jen, and have contributed greatly in all fields in our society. Their outstanding performance serves as the cornerstone of the university's future development. Currently, Fu Jen provides 11 colleges with 48 departments, 47 master program, 23 in-service master program, 11 doctoral program, and also 16 departments in School of Continuing Education. There are seven goals of Fu Jen, such as human dignity, meaning of life, academic research, community awareness, dialogue with cultures, religious cooperation, and spirit of service.

Figure 1. Logo of Fu Jen Catholic University Taiwan

2.2. Department of Food Science

In 1963, the Department of Family Studies and Nutrition Sciences was established and grouped into the Family Studies section and the Nutrition Sciences section. Nutrition Sciences section was combined with the Food Science section as the Department of Nutrition and Food Science in 1971. The Graduate Institute of Nutrition and Food Science was established and started to offer a Master's degree program in 1983. The Doctoral program was joined to the Institute in 1995. Food Science section became an individual department in 2006 which offers Bachelor's degree program and Master's degree program until now. The Departement of Food Science have a mission to promote the healthier, tastier and safer food for improving food quality, human health, and wellness.

3. RESEARCH PROJECT

3.1. Background of Research

Mung bean is one of a plant that classified in legume that have a short growing period in short season (Yao *et al*., 2008). Growth of Mung bean spread in South Asia, East Asia, and Southeast Asia. Mung bean have a many important compound and also bioactive compound. The important compound like vitamin B1, Thiamin, and niacin is available in mung bean (Shi *et al*., 2016). Besides that, mung bean contain a protein and essential amino acid that is lysine. Besides have a important compound, Mung bean also have a bioactive compound like antioxidant, antitumor, and antidiabetic (Xue *et al*., 2016). Mung beans also have a phenolic compound and flavonoid and this two components have a correlation with antioxidant activity in mung bean (Shi *et al*., 2016). Moreover there are nonphenolic compound that also have a correlation with antioxidants like tocopherols, carotenoids, and saponins (Anwar *et al*., 2007). Antioxidants include the important of bioactive compound. Antioxidants can prevent from risk of chronic disease like cancer and cardiovascular disease (Bielsaski *et al*., 2009).

In this century, people also process the raw mung bean and make into porridge, soup, salad, and starch to making a noodle. Eventough, raw mung bean have a antioxidants, but germinated mung bean have a higher antioxidants than raw mung beans. Antioxidants in mung bean is available in the form of vitamin C, whereas vitamin C is a best antioxidant (Guo *et al*., 2012). Germination is a best ways to improve the bioactive compounds because from a economic point of view and effectiveness there were cheap and have a short time to germinate mung bean (Mbithi *et al*., 2000). During germination process, carbohydrate broke down because of enzymatic activity and generate the production of polyphenols (Chon, 2013). Germination have a similar method with fermentation. The difference is fermentation use some additivies to breakdown complex compound into simple compound, while germination occurs naturally.

There were some literature that shown the data that time of germination affect in antioxidant activity. Antioxidant activity have a optimum time in germination. The longer germination period will increase the antioxidant activity, but if germination time over than 4 days the antioxidant activity will decrease (Xue *et al*., 2016). Moreover, cooking process also affect in antioxidant activity and total phenol contents. Processing methods of legumes like mung bean will increase bioavailabilty of nutrient, inactivating antinutient, increase inhibitor, and also increase antioxidant activity and total phenol contents. Processing methods like cooking not only affect the appearance of food, but also affect in bioactive compound and other component in food (Xu *et al*., 2008). Based on journal written by (Ismail *et al*., 2004) soaking and thermal cooking will decrease total phenol contents because phenol compound will breakdown and leaching in water. Boiling and boiling with pressure also decrease the radical scavenging activity, but pressure boiling decrease more radical scavenging activity. In another journal written by (Krishnaiah *et al*., 2013) Therefore, the purpose of this research is to know the effect of of different cooking process (Boiling and sous vide) on the total phenol contents and radical scavenging activity of Indonesia and Taiwan mung bean. The hypothesis are whether total phenolic contents and radical scavenging activity will decreased and breakdown while the temperature was increased. The recent journal already alludes of this topic but not complete yet. For example; (Xu *et al*., 2008) research only use raw mung bean sample, (Xue *et al*., 2016) research only observed antioxidant and bioactive compound in raw mung bean and germinated mung bean without cooking treatment.

3.2. Literature Review

3.2.1. Mung Bean

Mung bean classified in legume, that the growth distribution spread in most asia continent. Mung Bean classified into angiosperm dicot corps and classified in Fabaceae family, the color is green, but it can be yellow or tawny brown (Bailey, 1970) and (Degefa, 2016). Mung bean has found firstly in India in ancient era. Mung Bean can only growth in tropical and subtropical region. Besides India, Mung Bean also discovered in Malaysia, Indonesia, China, Taiwan, Myanmar, Thailand, South America, and Africa in tropical region (Lawn and Russel, 1978). Recent study show that Mung Bean can growth in lower, drier, and warmer area and the growth distribution spread

widely (Degefa, 2016). Mung Bean (*Vigna Radiata*) have a many beneficial component such as protein, low saturated fat, niacin, iron, magnesium, vitamin K, vitamin C, and vitamin B6 (Singh *et al*., 2013). Based on data, mung bean contains 240 g/kg proteins, 630 g/kg carbohydrates, essential amino acid that is lysine 504 mg/g (Anwar *et al*., 2007). Moreover, Mung Bean also have a many bioactive compound like polyphenols, anti-inflammatory, antidiabetic, antitumor, and antioxidants (Kanatt *et al*., 2011). Mung Bean have a many beneficial for health, it can solve gastrointestinal problem because mung bean have good disgetibility and it can solve the problem of chronic disease like cancer and cardiovascular disease (Min, 2001) and (Singh *et al*., 2013).

3.2.2. Sample Preparation

Sample preparation in this research is divide into 3 part, there are solvent extraction, evaporation, and freeze drying. Extraction has a purpose to obtain the components which are desired. Extraction is the method to separate compound from the matrix. Compound will dissolved into solvent if it has a same polarity. Sample extraction for Mung Bean is to obtain phenolic acid, where phenolic acid will measured as total phenol compound and antioxidant. Extraction of the phenolic acid using a solvent extraction method or liquid-liquid extraction (Lee *et al*., 2000). Based on (Singh *et al*., 2013), (Fidrianny *et al*., 2014), (Shi *et al*., 2016), and (Xue *et al*., 2016) journals, solvent that used to extraction mung bean are methanol, n-hexane, acetone, and ethanol.

After extraction, mung bean sample enter at the evaporation stage. Purpose of evaporation is to evaporate the solvent so, the crude extract will obtained. Rotary evaporator is the one of instrument support to evaporated sample. The principal work of rotary evaporator are decrease the boiling point of the solvent by reduce the pressure with rotating the solvent to optimizing the evaporation. Sample was rotating and heating at the same time. First, Rotary evaporator was set the pressure in accordance with the solvent that will extracted. Then, the pressure was reduced slowly. While the pressure was reduced, the solvent will lead to condensor in vapor form because the boiling point already appropriate with solvent boiling point. In condensor, vapor will converted into liquid form and received by collection flask.

After evaporation, the sample will freeze dried. The aim of freeze drying is to changes the form of extract mung bean. While, after evaporation extract mung bean still sticky and hard to analyze. So freeze drying will change into powder. The principle of freeze drying is to dry the solvent that still in sample extract. The solvent will freeze first and then dried.

3.2.3. Total Phenolic Contents

Phenolic is a compound that classified in antioxidant class. Phenolic also affect the antioxidant activity. There are many compound that classified into phenolic compound such as tannin, flavonoid, quinone, anthocyanin, anthocyanidin, lignin, fenolic acid, and tannin. Flavonoid is a compound that have a highest antioxidant than phenolic acid. Total Phenolic Content can measured using Folin- Ciocalteau phenol. This reagent contain phosphomolybdic – phosphotungstic acid (Mo^{6+}). Phenolic compound have a hydroxyl group. While this analysis done to obtain ability of phenolic compound that have a hydroxyl group to transfer electron to phosphomolybdic – phosphotungstic acid. This reaction will show change of the color of the sample into blue color. Folin-Ciocalteau have a absorbance value in range 500 nm -750 nm. Analysis of total phenolic Content using a gallic acid as a standard (Singleton et al., 1965).

3.2.4. Radical Scavenging Activity

Free radical is the product that have a dangerous action in the body. This product is a result from metabolism system in the body. Free radical can cause chronic disease like cancer, cardiovascular disease, diabetes, mutation, and until Alzheimer symptoms. (Christen, 2000) and (Giugliano, 1996). Free radical in the chemical structure have a electron pair but one of electron have not a pair, so this electron can be told as free radical. Antioxidant is the compound that can prevent free radical to cause chronic disease. Antioxidant will donate electron to make a free radical have a electron pair at all or become stable. Antioxidant will work as stabilizer. There were many method to analyze antioxidant. Method that often used is DPPH radical scavenging activity. While DPPH reagent added into sample the color while change from deep purple into yellow. DPPH reagent have a sensitivity against light, so must store in dark place. The

measurement of antioxidant related with IC_{50}. IC_{50} is a the effectiveness of antioxidant to inhibit at least 50% at a certain concentration (Williams et al., 1995)

4. RESEARCH METHODOLOGY

4.1. Material

4.1.1. Tools

Rice cooker, sous vide, bowl, wood hammer, refrigerator, incubator, sample, vacuum bags, packaging machine, plastic wrap, blender, rotary evaporator, freeze drying, microcentrifuge tube 50 ml, microcentrifuge tube 1.5 ml, dropper pipette, micropipette, white tips, yellow tips, spoon ,mortar, pestle, analytical balance, beaker glass, sample mixer, aluminum foil, centrifuge, erlenmeyer flask, clamp, rubber bands, rubber cover, buffering tubes, collection bottle, timer, vortex, freezer, dry bath incubator, microcentrifuge racks, 96 well plate, and microplate reader.

4.1.2. Ingredients

Mung bean seeds (*Vigna radiata L.*), mineral water, mung bean powder, DD (Distilled Deionized) water, Ethanol 70%, Methanol, DPPH powder, gallic acid, Folin-Ciocalteu phenol, and Na_2CO_3 15%.

4.2. Methods

4.2.1. Mung Beans variety

Two variety mung bean are used in this research project. There are Indonesia's mung bean and Taiwan's mung bean bought from Carrefour in the New Taipei City, Taiwan.

Figure 2. Taiwan Mung Bean

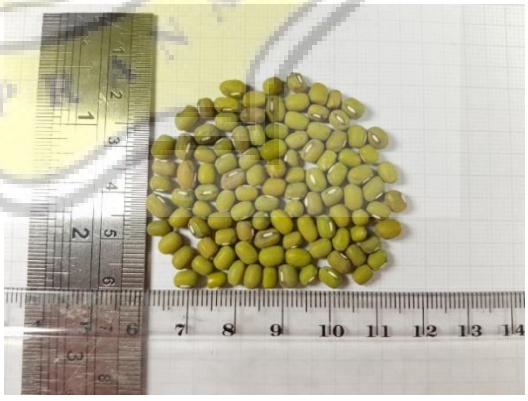


Figure 3. Indonesia Mung Bean

4.2.2. Mung Beans Pre-treatment

Two different pre-treatment were used for the mung beans sample used in this project; soaking and germination. For the soaking pre-treatment, 800 grams mung beans from each variety were weighed and washed using water for 3 times. Then, put into bowl and weighed. After that, mineral water were added. The mung beans were soaked in mineral water and were stored for 24 h at 4°C. For the germination pre-treatment, 800 grams amount of mung beans for each variety were weighed and washed using water for 3 times. Then, put in the bowl and weighed. After that, mineral water were added and mung beans were germinated in the incubator at 32°C for 24 h.

Figure 4. Soaking Pre-treatment

Figure 5. Germination Pre-treatment

4.2.3. Mung Bean Cooking Treatment

Two different cooking treatment were used for the mung beans sample in this project; boiling and *sous vide*. For the boiling treatment, soaked and germinated mung beans were directly cooked in boiling water at 100^{o}C in a rice cooker using a seed-to-water ratio of 1:2. Samples were cooked by rice cooker for 40 minutes and stew for 20 minutes. For the *sous vide* (SV) treatment, the processing was carried out on each mung beans and were transferred into vacuum bags and sealed with packaging machine and cooked for 5 h in a pot at 80°C for each different of mung bean's variety.

Figure 6. Boiling Cooking Treatment

Figure 7. Sous Vide Cooking Treatment

4.2.4. Extraction Mung bean sample

Two different solvent were used for mung beans sample in this project; ethanol 70% and DD water. Raw mung beans were pounded in mortar using pestle. After that, weighed as much as 5 grams using analytical balance. Then, 50 ml ethanol 70% were added. Then, solution were mixed with sample mixer during 2 h. Then, solution were centrifuged at 1000 rpm for 10 minutes. After that, supernatant were collected, covered with aluminum foil, and stored in freezer. For water extract has similar ways with ethanol extract but stored in refrigerator. After that, mung bean sample were evaporated using rotary evaporator, and then evaporated sample were dried using freeze drying for 2 days. After 2 days, mung bean sample transformed into powder and stored in freezer for further analysis.

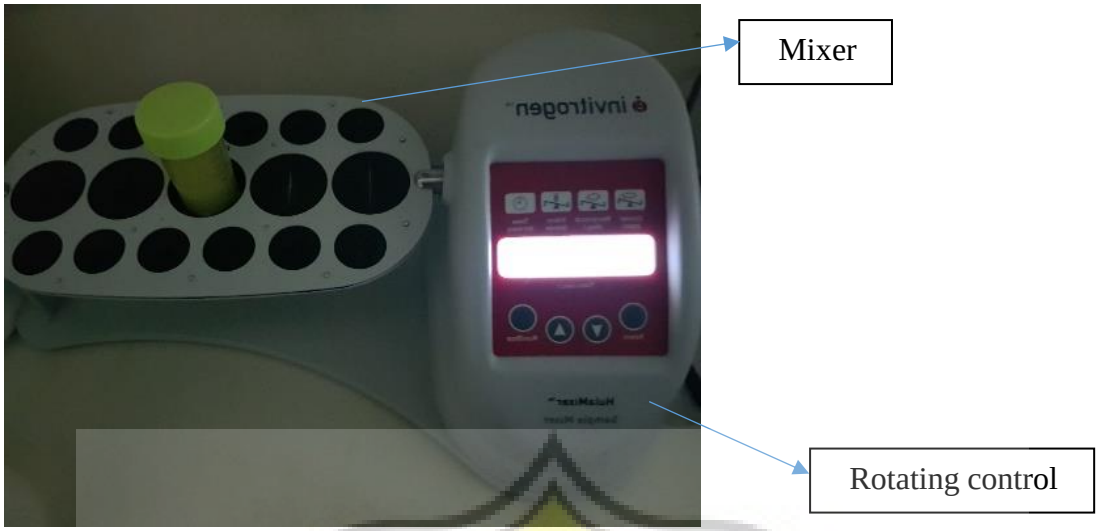


Figure 8. Appearance Sample Mixer

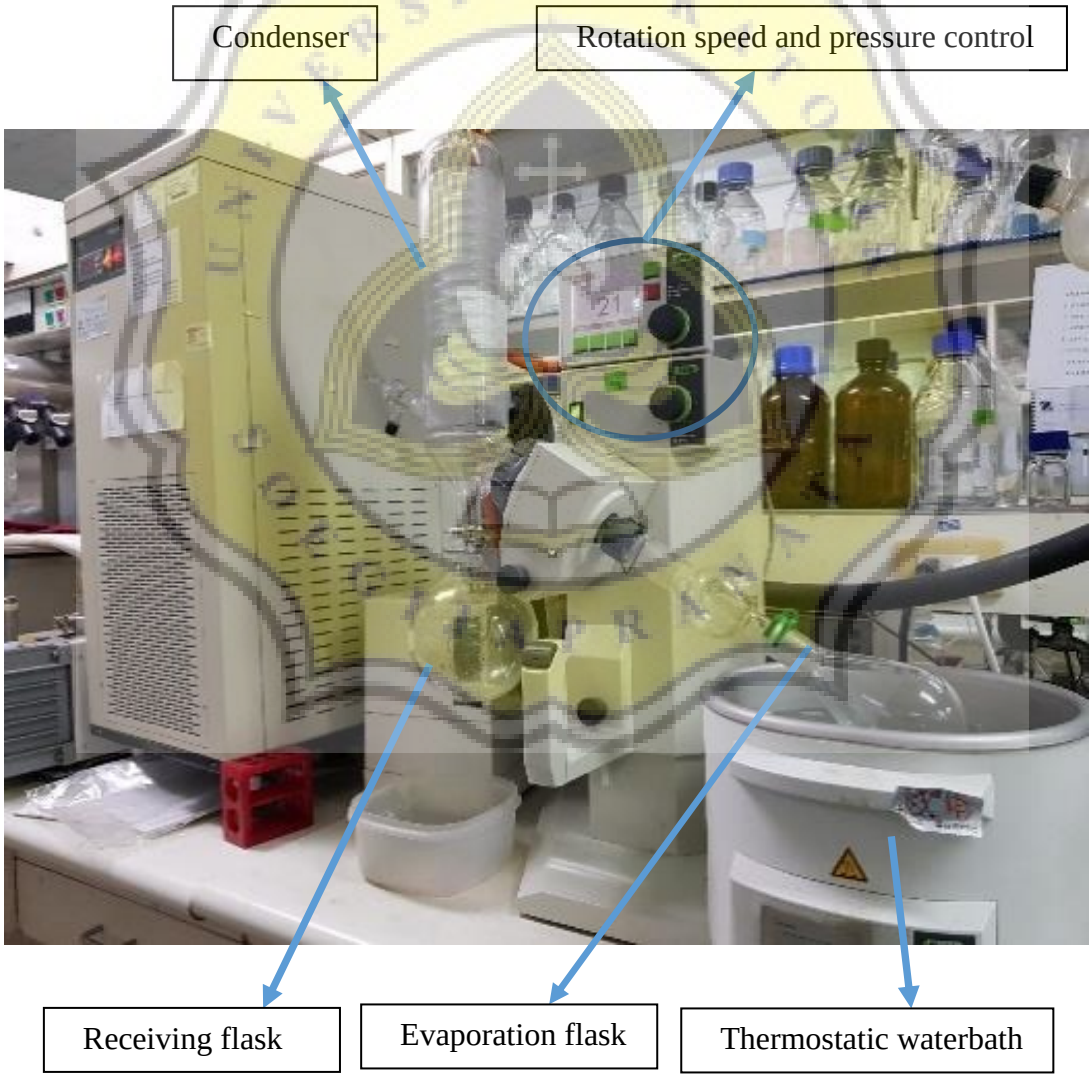


Figure 9. Appearance Rotary Evaporator

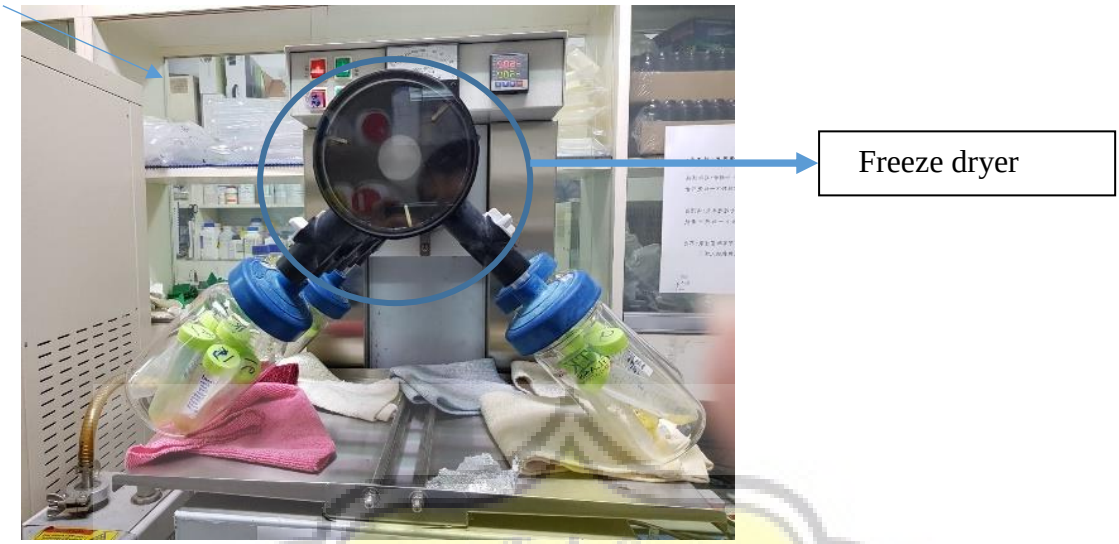


Figure 10. Appearance Freeze Dryer

4.2.5. DPPH Radical Scavenging Activity

4.2.5.1. Preparation DPPH solution (1 mM)

3.94 mg DPPH powder were weighed and dissolved in 1 ml methanol. From that solution the concentration is 10 mM. Then, from that solution 100 µl were taken and dissolved into 900 µl methanol. From that solution the concentration is 1 mM and used for DPPH analysis.

4.2.5.2. Analysis

Mung beans sample were dissolved into 1 ml ethanol 70%/ DD water. Then, mix. After that, solution were taken as much 160 µl using micropipette and injected in 96 well plate. Then, 40 µl DPPH solution were added. After that, solution were kept in the dark place in room temperature (30^{O}C) for 30 minutes. After 30 minutes, absorbance were measured with wavelength 517 nm using microplate reader. For control group, 160 µl solvent added with 40 µl DPPH solution and kept in the dark place in room temperature (30^{O}C) for 30 minutes and the absorbance value were measured with wavelength 517 nm. For blank group, 160 µl sample added with 40 µl solvent and and kept in the dark place in room temperature (30^{O}C) for 30 minutes and the absorbance value were measured with wavelength 517 nm using microplate reader. For sample and control

group measurement of absorbance were done 3 times. After that, DPPH scavenging activity was calculated with equation.

$$\text{DPPH scavenging activity (\%)} = \frac{\text{A control} - (\text{A sample} - \text{A blank})}{\text{A control}} \times 100\%$$

4.2.6. Total Phenolic Content

4.2.6.1. Create standard curve

1 grams gallic acid was dissolved into 1 ml DD water. Then, diluted into concentration 10, 30, 50, 100, and 200 µl/ ml. Then from each concentration, taken 30 µl and added with 120 µl folin-ciocalteu phenol. After that, mix and keep in the dark place for 5 minutes. And then, 600 µl Na_2CO_3 was added and react for 30 minutes in temperature 30^{O}C. After 30 minutes, solution was centrifuged at 6600 x g for 1 minutes. Then, collect the supernatant and take 200 µl supernatant using micropipette and injected into 96 well plate. Then absorbance was measured. For standard curve, plot absorbance value as Y axis and concentration as X axis. Then make a graph and equation.

The standar curve of total phenolic contents are shown in figure 11.

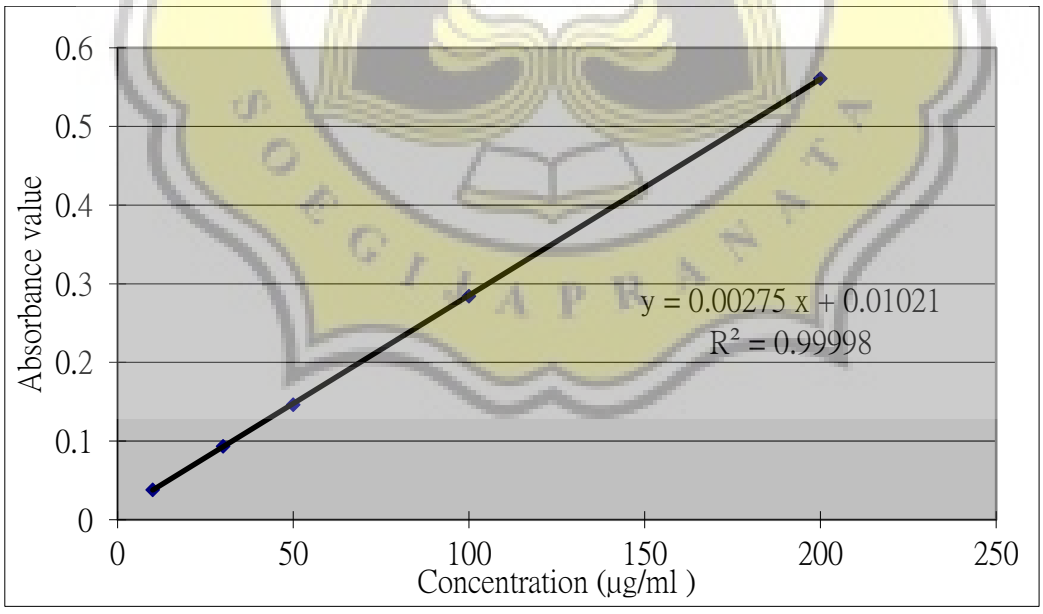


Figure 11. Standard Curve of Gallic Acid

4.2.6.2. Analysis

Mung bean sample was dissolved into 1 ml solvent. Then, solution was taken 30 µl and added with 120 µl folin-ciocalteu phenol. After that, mix and keep in the dark place for 5 minutes in dry bath incubator. And then, 600 µl Na_2CO_3 was added and react for 30 minutes in temperature 30°C. After 30 minutes, solution was centrifuged at 6600 x g for 1 minutes. Then, collect the supernatant and take 200 µl supernatant using micropipette and injected into 96 well plate. Then absorbance was measured with wavelength 750 nm using microplate reader. For blank group, 30 µl sample and added with solvent. Then 600 µl Na_2CO_3 was added and react for 30 minutes in temperature 30°C. After 30 minutes, solution was centrifuged at 6600 x g for 1 minutes. Then, collect the supernatant and take 200 µl supernatant using micropipette and injected into 96 well plate. Then absorbance was measured with wavelength 750 nm using microplate reader. After that, total phenol content was calculated using equation from standard curve.

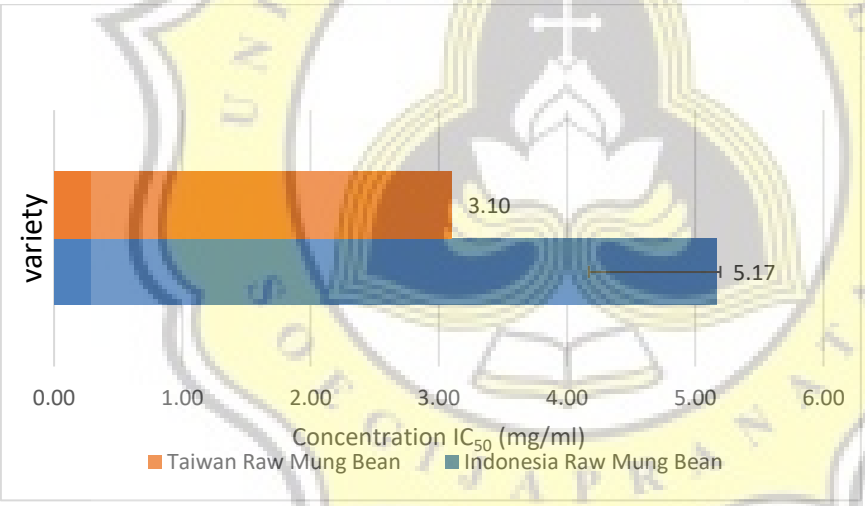
4.2.7. Statistical Analysis

Data was organize by Microsoft Excel statistical analysis program.

5. RESULT AND DISCUSSION

Mung bean have a many important compound and bioactive compound that contained in there. Mung bean contain carbohydrates, vitamin ; such as thiamin, riboflavin, vitamin C, niacin, vitamin E, vitamin K, and folate; protein, and mineral such as Ca, Mg, Fe, P, and Zn. Moreover Mung Bean contain a bioactive compound like antioxidants, flavonoid, phenolic compound, and nonphenolic compound such as carotenoid, tocopherols, and saponin. Germination is a one of method to increase the bioactive compound in mung bean. There are a some enzymatic change that change from carbohydrate into polyphenols. Soaking of mung bean is affect the bioactive compound have decrease because the bioactive compound like phenolic acid will breakdown and leacing in water.

5.1. Radical Scavenging Activity

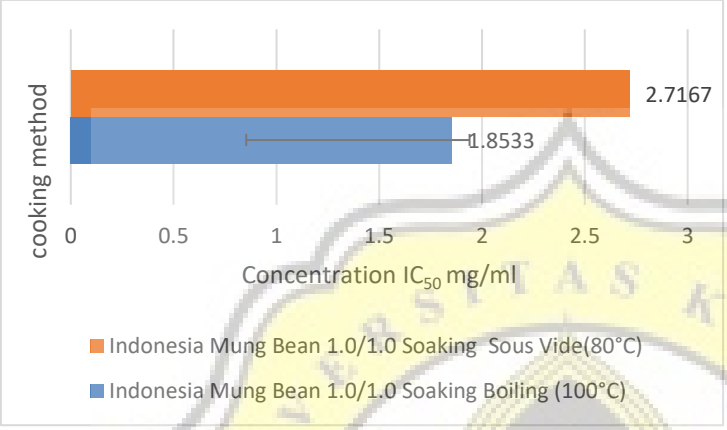


Graph 1. Radical Scavenging Activity Indonesia and Taiwan raw mung bean

Graph 1. shows the radical scavenging activity on Indonesia and Taiwan raw mung bean which have not been treated. The results are Indonesia raw mung bean have a higher concentration on radical scavenging activity. The radical scavenging activity was influenced by concentration of IC_{50}. The lower concentration of IC_{50} will give a higher radical scavenging activity. So it can be concluded that Taiwan mung bean has a higher radical scavenging activity. Mung bean have a much of variety with have a different characteristic. But one variety with other variety have a different quantity of compound. There were some factor that influenced of different amount of compound such as soil

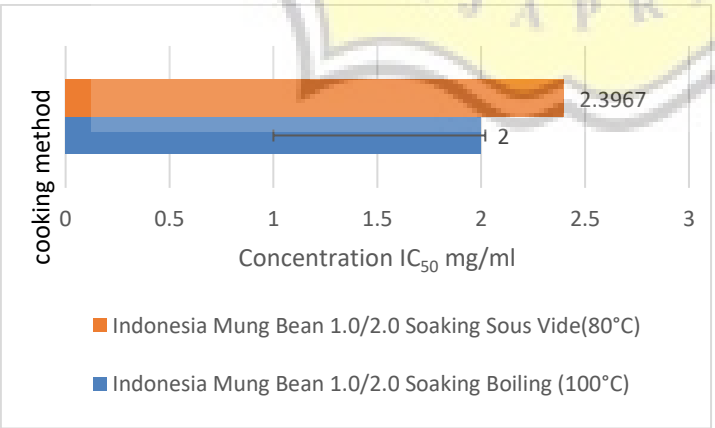
conditions, maturity of mung bean while harvested, and treatment after post-harvest. (Anwar *et al*., 2007). The climate also influenced the compound of mung bean, Indonesia in tropical region but Taiwan in sub-tropical region. Moreover the mung bean that's on this research taken with randomly variety.

5.1.1. Indonesia Mung Bean



Graph 2. Radical Scavenging Activity comparing cooking method Indonesia Soaking Mung Bean

Graph 2. shows comparing the cooking method on the radical scavenging activity of Indonesia soaking Mung Bean. Cooking method using boiling method result the lower concentration IC_{50} than cooking method using sous vide method in all different ratio water added. The radical scavenging activity was influenced by concentration of IC_{50}. The lower concentration of IC_{50} will give a higher radical scavenging activity. So, it can be concluded cooking method using boiling method result highest radical scavenging activity.



Graph 3. Radical Scavenging Activity comparing cooking method Indonesia Soaking Mung Bean

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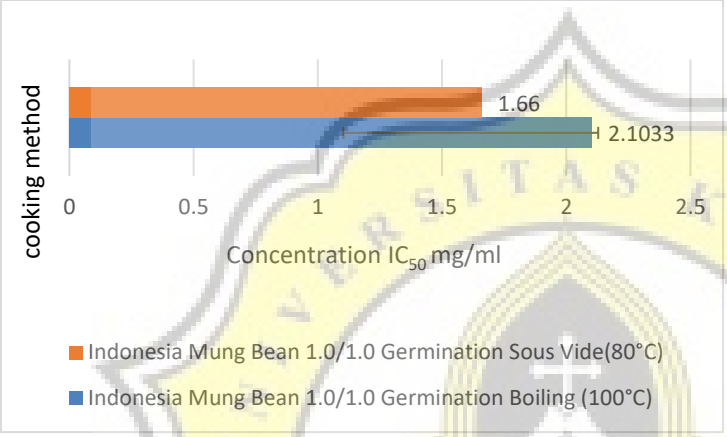
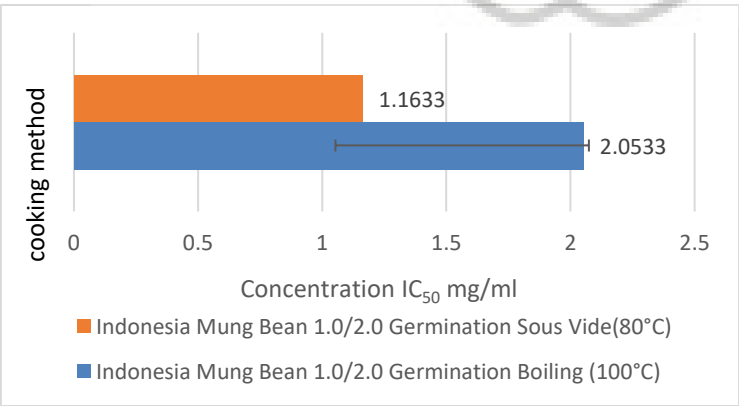


Graph 4. Radical Scavenging Activity comparing cooking method Indonesia Germination Mung Bean

Graph 4. shows comparing the cooking method on the radical scavenging activity of Indonesia germination Mung Bean. Cooking method using sous vide method result the lower concentration IC_{50} than cooking method using boiling method in all different ratio water added. The radical scavenging activity was influenced by concentration of IC_{50}. The lower concentration of IC_{50} will give a higher radical scavenging activity. So, it can be concluded cooking method using sous vide method result highest radical scavenging activity.

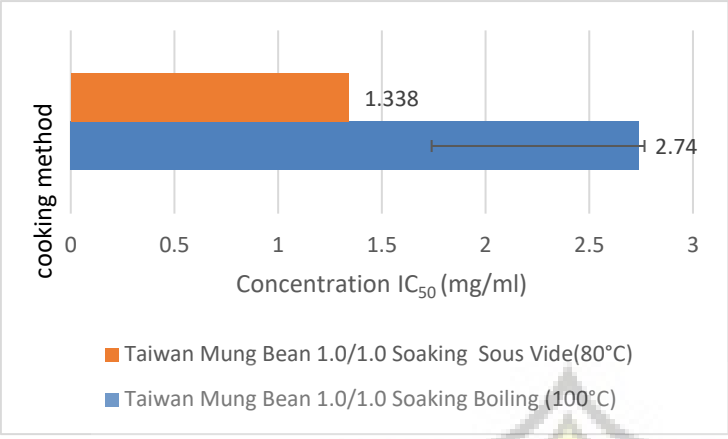


Graph 5. Radical Scavenging Activity comparing cooking method Indonesia Germination Mung Bean

Graph 5. shows comparing the cooking method on the radical scavenging activity of Indonesia germination Mung Bean. Cooking method using sous vide method result the lower concentration IC_{50} than cooking method using boiling method in all different ratio water added. The radical scavenging activity was influenced by concentration of IC_{50}. The lower concentration of IC_{50} will give a higher radical scavenging activity. So, it can be concluded cooking method using sous vide method result highest radical scavenging activity.

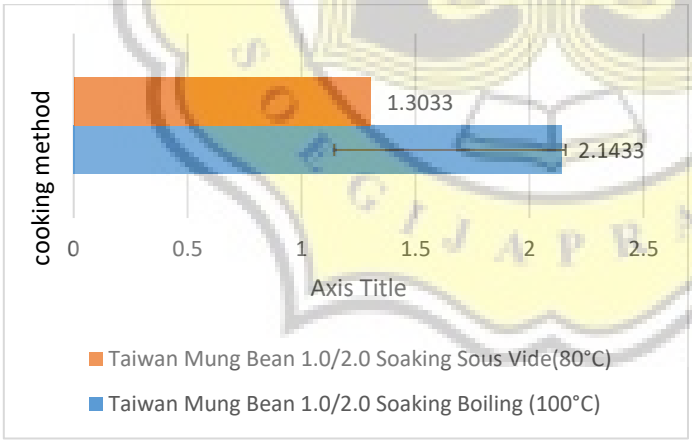
From the graph 2 to graph 5, it can be concluded that cooking method with sous vide and pre-treatment using germination is the combination of treatment that results lowest concentration of radical scavenging activity of Indonesia mung bean. The radical scavenging activity was influenced by concentration of IC_{50}. The lower concentration of IC_{50} will give a higher radical scavenging activity. Based on journal written by (Xue *et al*., 2016) germination method will increased the antioxidant activity. (Krishnaiah *et al*., 2013) also said that radical scavenging activity will increase until temperature 60ºC and after that will decreased because antioxidant can easily denaturated in high temperature. Germination also can improve bioactive compound like antioxidant because there were a secondary metabolite, whereas result of aerobic respiration and metabolism during germination. So, produce a phenolic, flavonoid, and tocopherols. Germinated mung bean can be as functional food because nutrition value were enhanced and increased and the also increased the effect of health promote. (Ganesan *et al*., 2018). So that's why the germination pre-treatment and sous vide cooking method result the higher antioxidant activity in Indonesia mung bean. But based on journal written by (Abacan *et al*., 2016) radical scavenging activity will decreased on temperature 75ºC and will increased on temperature 100ºC. This different result will discuss for furtherwork research with method validation to make sure that the data is valid. There are no effect of adding water with different ratio on radical scavenging activity.

5.1.2.Taiwan Mung Bean



Graph 6. Radical Scavenging Activity comparing cooking method Taiwan Soaking Mung Bean

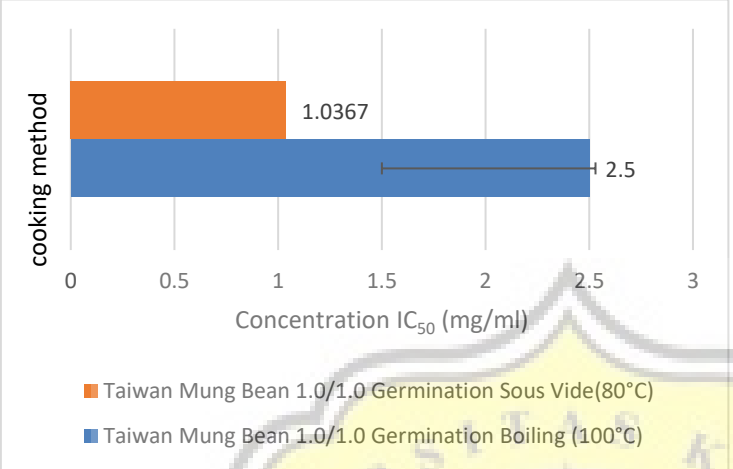
Graph 6. shows comparing the cooking method on the radical scavenging activity of Taiwan soaking Mung Bean. Cooking method using sous vide method result the lower concentration IC_{50} than cooking method using boiling method in all different ratio water added. The radical scavenging activity was influenced by concentration of IC_{50}. The lower concentration of IC_{50} will give a higher radical scavenging activity. So, it can be concluded cooking method using sous vide method result highest radical scavenging activity.



Graph 7. Radical Scavenging Activity comparing cooking method Taiwan Soaking Mung Bean

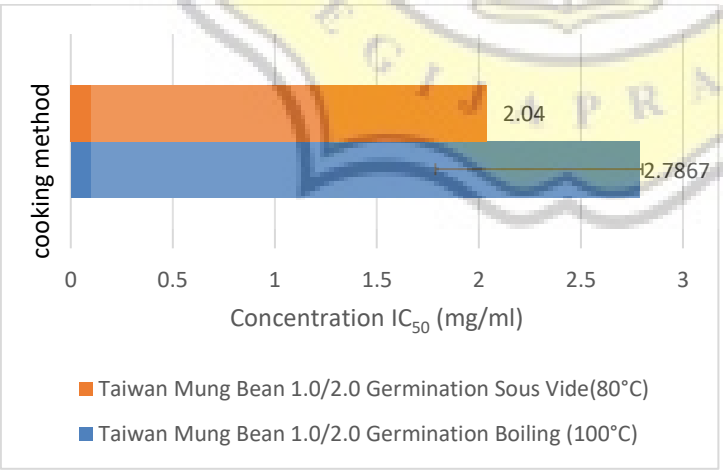
Graph 7. shows comparing the cooking method on the radical scavenging activity of Taiwan soaking Mung Bean. Cooking method using sous vide method result the lower concentration IC_{50} than cooking method using boiling method in all different ratio water added. The radical scavenging activity was influenced by concentration of IC_{50}. The

lower concentration of IC_{50} will give a higher radical scavenging activity. So, it can be concluded cooking method using sous vide method result highest radical scavenging activity.



Graph 8. Radical Scavenging Activity comparing cooking method Taiwan Germination Mung Bean

Graph 8. shows comparing the cooking method on the radical scavenging activity of Taiwan germination Mung Bean. Cooking method using sous vide method result the lower concentration IC_{50} than cooking method using boiling method in all different ratio water added. The radical scavenging activity was influenced by concentration of IC_{50}. The lower concentration of IC_{50} will give a higher radical scavenging activity. So, it can be concluded cooking method using sous vide method result highest radical scavenging activity.

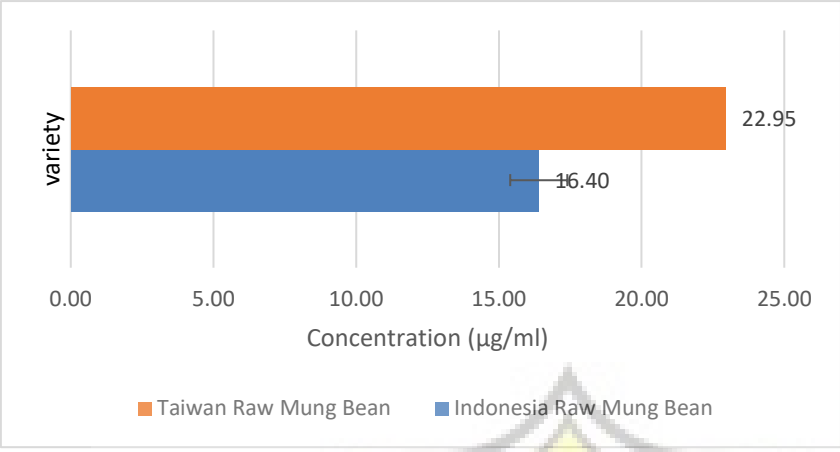


Graph 9. Radical Scavenging Activity comparing cooking method Taiwan Germination Mung Bean

Graph 9. shows comparing the cooking method on the radical scavenging activity of Taiwan germination Mung Bean. Cooking method using sous vide method result the lower concentration IC_{50} than cooking method using boiling method in all different ratio water added. The radical scavenging activity was influenced by concentration of IC_{50}. The lower concentration of IC_{50} will give a higher radical scavenging activity. So, it can be concluded cooking method using sous vide method result highest radical scavenging activity.

From the graph 6 to graph 9, it can be concluded that cooking method with sous vide and pre-treatment using germination is the combination of treatment that results lowest concentration of radical scavenging activity of Taiwan mung bean. The radical scavenging activity was influenced by concentration of IC_{50}. The lower concentration of IC_{50} will give a higher radical scavenging activity. Based on journal written by (Xue *et al*., 2016) germination method will increased the antioxidant activity. (Krishnaiah *et al*., 2013) also said that radical scavenging activity will increase until temperature 60ºC and after that will decreased because antioxidant can easily denaturated in high temperature. Germination also can improve bioactive compound like antioxidant because there were a secondary metabolite, whereas result of aerobic respiration and metabolism during germination. So, produce a phenolic, flavonoid, and tocopherols. Germinated mung bean can be as functional food because nutrition value were enhanced and increased and the also increased the effect of health promote. (Ganesan *et al*., 2018). So that's why the germination pre-treatment and sous vide cooking method result the higher antioxidant activity in Taiwan mung bean. But based on journal written by (Abacan *et al*., 2016) radical scavenging activity will decreased on temperature 75ºC and will increased on temperature 100ºC. This different result will discuss for furtherwork research with method validation to make sure that the data is valid. There are no effect of adding water with different ratio on radical scavenging activity.

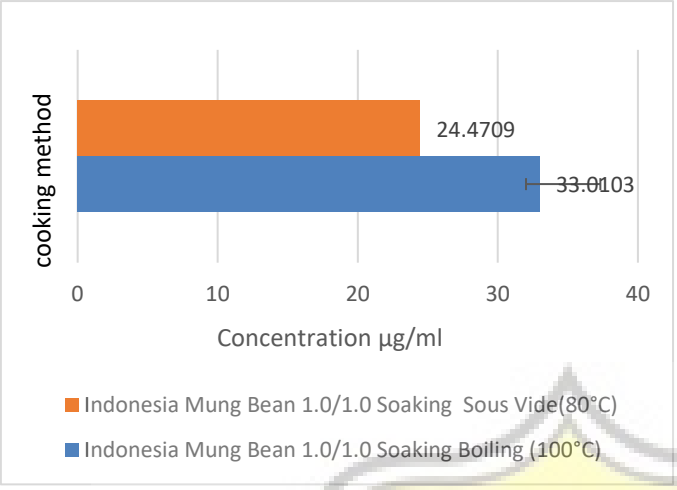
5.2. Total Phenol Contents



Graph 10. Total Phenol Contents Indonesia and Taiwan Raw Mung Bean

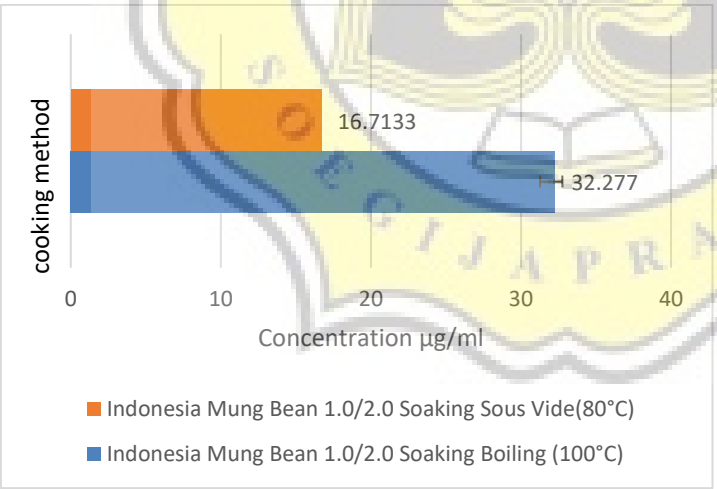
Graph 10. shows the total phenol content on Indonesia and Taiwan raw mung bean which have not been treated. The results are Taiwan raw mung bean have a higher concentration on total phenol contents. The total phenol contents was influenced by concentration. The higher concentration will give a higher total phenol content. So it can be concluded that Indonesia mung bean has a higher total phenol content. Mung bean have a much of variety with have a different characteristic. But one variety with other variety have a different quantity of compound. There were some factor that influenced of different amount of compound such as soil conditions, maturity of mung bean while harvested, and treatment after post-harvest. (Anwar *et al*., 2007). The climate also influenced the compound of mung bean, Indonesia in tropical region but Taiwan in sub-tropical region. Moreover the mung bean that's on this research taken with randomly variety.

5.2.1. Indonesia Mung Bean



Graph 11. Total Phenol Contents comparing cooking method Indonesia Soaking Mung Bean

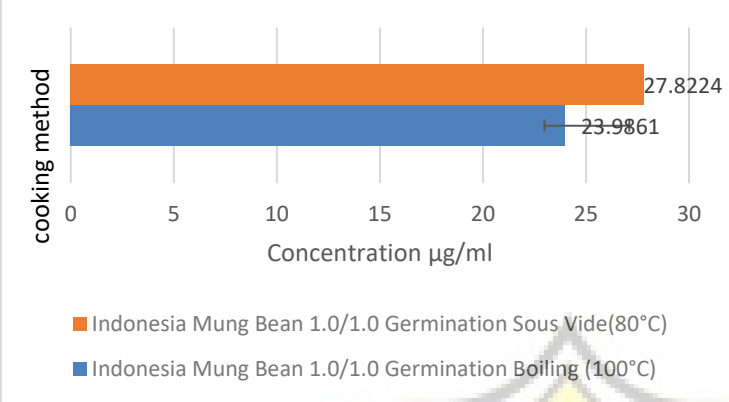
Graph 11. shows comparing the cooking method on the total phenol contents of Indonesia soaking Mung Bean. The total phenol contents was influenced by concentration. The higher concentration will give a higher total phenol content. Cooking method using boiling method result the higher concentration than cooking method using sous vide method in all different ratio water added. So, it can be concluded cooking method using boiling method result highest total phenolic contents.



Graph 12. Total Phenol Contents comparing cooking method Indonesia Soaking Mung Bean

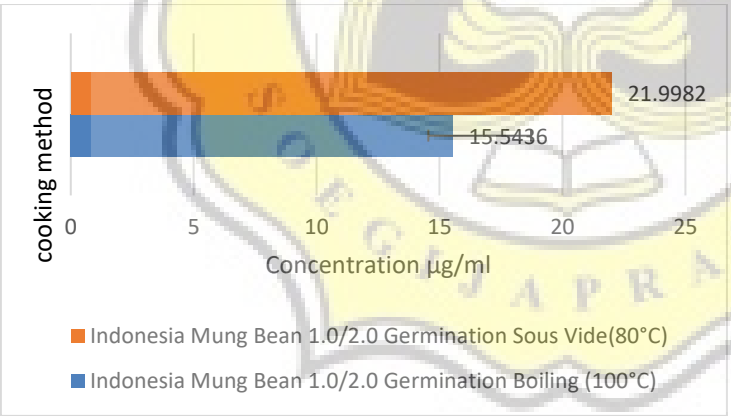
Graph 11. shows comparing the cooking method on the total phenol contents of Indonesia soaking Mung Bean. The total phenol contents was influenced by concentration. The higher concentration will give a higher total phenol content. Cooking method using boiling method result the higher concentration than cooking method using sous vide

method in all different ratio water added. So, it can be concluded cooking method using boiling method result highest total phenolic contents.



Graph 13. Total Phenol Contents comparing cooking method Indonesia germination Mung Bean

Graph 13. shows comparing the cooking method on the total phenol contents of Indonesia germination Mung Bean. The total phenol contents was influenced by concentration. The higher concentration will give a higher total phenol content. Cooking method using boiling method result the higher concentration than cooking method using sous vide method in all different ratio water added. So, it can be concluded cooking method using sous vide method result highest total phenolic contents.



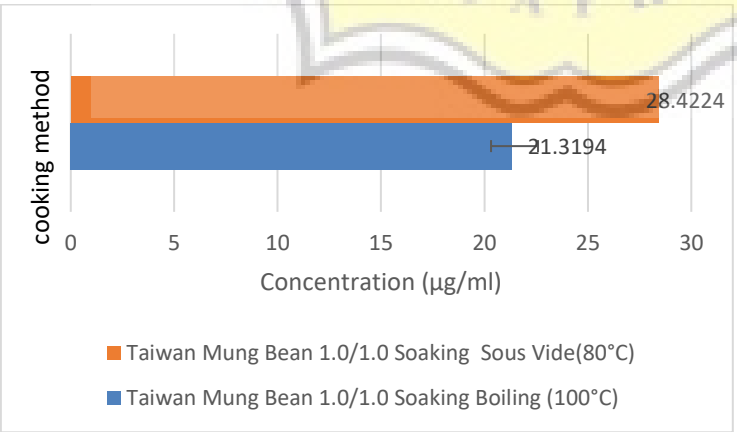
Graph 14. Total Phenol Contents comparing cooking method Indonesia germination Mung Bean

Graph 14. shows comparing the cooking method on the total phenol contents of Indonesia germination Mung Bean. The total phenol contents was influenced by concentration. The higher concentration will give a higher total phenol content. Cooking method using boiling method result the higher concentration than cooking method using sous vide

method in all different ratio water added. So, it can be concluded cooking method using sous vide method result highest total phenolic contents.

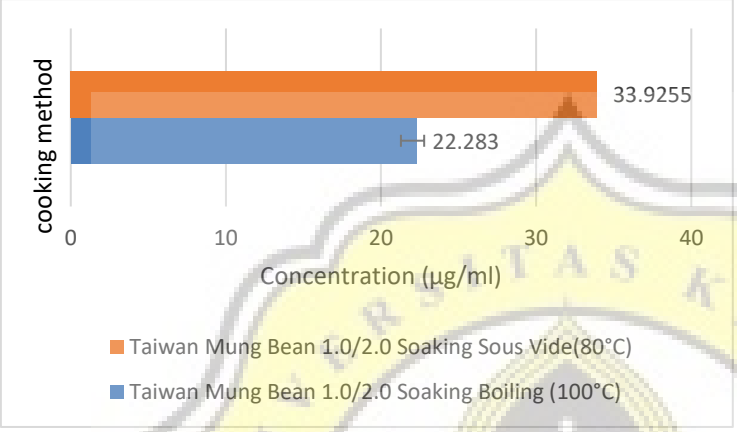
From the graph 11 to graph 14, it can be concluded that cooking method with boiling and pre-treatment using soaking is the combination of treatment that results higher concentration of total phenolic contents for Indonesia mung bean. The total phenolic contents was influenced by concentration. The higher concentration will give a higher total phenolic contents also. Based on journal written by (Xu *et al*., 2008) germination method will increased the total phenol contents. (Krishnaiah *et al*., 2013) also said that total phenolic contents will increase until temperature 60ºC and after that will decreased. (Abacan *et al*., 2016) also said that total phenolic contents will decreased along with the increase of temperature because phenolic is volatile compound and volatile compound have a sensitivity with high temperature and the high temperature will breakdown bound of phenolic. So based on the journal if correlated with methodology can concluded that sous vide have a longer time of cooking than boiling, though the temperature was low but the longer time of cooking can breakdown the total phenolic contents. But if seen in term of method validation, it can be told that method validation not done in this research. A research which use standard curve should be done with method validation to make sure that the data is valid. For furtherwork research, research should be done with method validation to make sure the data is valid.

5.2.2. Taiwan Mung Bean



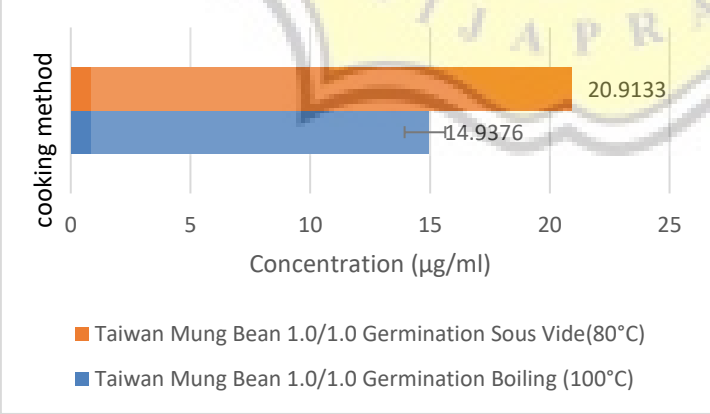
Graph 15. Total Phenol Contents comparing cooking method Taiwan soaking Mung Bean

Graph 15. shows comparing the cooking method on the total phenol contents of Taiwan soaking Mung Bean. The total phenol contents was influenced by concentration. The higher concentration will give a higher total phenol content. Cooking method using sous vide method result the higher concentration than cooking method using boiling method in all different ratio water added. So, it can be concluded cooking method using sous vide method result highest total phenolic contents.



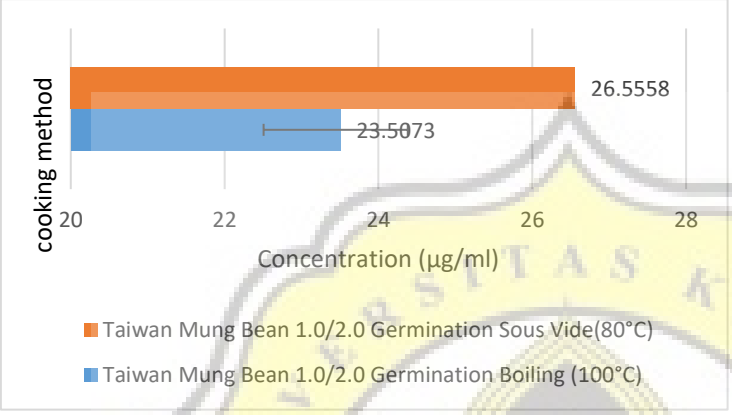
Graph 16. Total Phenol Contents comparing cooking method Taiwan soaking Mung Bean

Graph 16. shows comparing the cooking method on the total phenol contents of Taiwan soaking Mung Bean. The total phenol contents was influenced by concentration. The higher concentration will give a higher total phenol content. Cooking method using sous vide method result the higher concentration than cooking method using boiling method in all different ratio water added. So, it can be concluded cooking method using sous vide method result highest total phenolic contents.



Graph 17. Total Phenol Contents comparing cooking method Taiwan germination Mung Bean

Graph 17. shows comparing the cooking method on the total phenol contents of Taiwan germination Mung Bean. The total phenol contents was influenced by concentration. The higher concentration will give a higher total phenol content. Cooking method using sous vide method result the higher concentration than cooking method using boiling method in all different ratio water added. So, it can be concluded cooking method using sous vide method result highest total phenolic contents.



Graph 18. Total Phenol Contents comparing cooking method Taiwan germination Mung Bean

Graph 18. shows comparing the cooking method on the total phenol contents of Taiwan germination Mung Bean. The total phenol contents was influenced by concentration. The higher concentration will give a higher total phenol content. Cooking method using sous vide method result the higher concentration than cooking method using boiling method in all different ratio water added. So, it can be concluded cooking method using sous vide method result highest total phenolic contents.

From the graph 15 to graph 18, it can be concluded that cooking method with sous vide and pre-treatment using soaking is the combination of treatment that results higher concentration of total phenolic contents for Taiwan mung bean. The total phenolic contents was influenced by concentration. The higher concentration will give a higher total phenolic contents also. Based on journal written by (Xu *et al*., 2008) germination method will increased the total phenol contents. (Krishnaiah *et al*., 2013) also said that total phenolic contents will increase until temperature 60ºC and after that will decreased. (Abacan *et al*., 2016) also said that total phenolic contents will decreased along with the increase of temperature because phenolic is volatile compound and volatile compound have a sensitivity with high temperature and the high temperature will breakdown bound

of phenolic. But based on the journal (Xue *et al*., 2016) that the germination need to do until 4 days to get a higher total phenolic contents and this research only germinate the mung bean during 24h or 1 day. So that's why the total phenolic contents in Taiwan mung bean with pre-treatment using soaking have a higher total phenolic contents. But if seen in term of method validation can be told that method validation not done in this research. A research which use standard curve should be done with method validation to make sure that the data is valid. For furtherwork research, research should be done with method validation to make sure the data is valid.

6. CONCLUSION AND SUGGESTION

6.1. Conclusion

Pre-treatment using germination method and cooking using Sous Vide is the combination treatment that more effective to maintain antioxidant activity in Indonesia Mung Bean, this can be seen from graph 4 and graph 5. While pre-treatment using germination method and cooking using Sous Vide method is the combination treatment that more effective to maintain antioxidant activity in Taiwan Mung Bean, this can be seen from the graph 8 and graph 9. Then pre-treatment using soaking method and cooking using boiling is the combination treatment that more effective to maintain total phenol contents in Indonesia Mung Bean, this can be seen from graph 11 and graph 12. While pre-treatment using soaking method and cooking using sous vide is the combination treatment that more effective to maintain total phenol contens in Taiwan Mung Bean, this can be seen from graph 15 and graph 16

6.2. Suggestion

For advanced research, treatment with germination need to be done for several days and then the IC_{50} and Total Phenol Contents compared. Other than that, for next research treatment using fermentation can be done to compare with soaking and germination treatment method. And then total flavonoid and Ferric Reducing Antioxidant Power (FRAP) need to be done for advanced research. Moreover, research that using standard curve should be done with method validation.

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