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THE UTILIZATION OF VEGETABLE AND FRUIT WASTES FOR *SACCHAROMYCES CEREVISIEAE* CELL WALL BASED SS-GLUCAN PRODUCTION WITH ANTIOXIDANT ACTIVITY

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Abstract

The study aims to determine the number of mass and antioxidant activity of ß-glucan extracted from *S.cerevisieae* which grown on vegetable and fruit wastes. The method used is experimental with descriptive analysis which consisted of 3 treatments namely banana waste, papaya waste, and napa cabbage waste as fermentation medium, repeated thrice. Fermentation medium was made by mixing waste with water with the ratio of 1:2. Ten percent (w/v) of *S.cerevisieae* was inoculated and incubated for 48 hours, at 27 °C. Extraction of ß-glucan was carried out using acid-alkaline methods and antioxidant activity was tested by DPPH (2, 2-Dyphenyl-1-Picrylhydrayl) method and the microstructure of ß-glucan is determined by Scanning Electrone Microscope. The result showed that the best medium in producing ß-glucan was papaya waste which resulting 19.094 g ß-glucan mass, with radical scavenging activity of 20.71% and globular diameter of 533µm.

Keywords: antioxidant activity, ß-glucan, banana waste, napa cabbage waste, papaya waste

INTRODUCTION

Organic waste amounts up to 60% of total waste in Indonesia. According to the Directorate of Waste Management, total wastes in Indonesia are estimated to be 175,000 ton a day or 64 million ton per year (Yodha, 2018). This considerable amount of organic waste becomes one of the problems that is sufficiently disestablished by the government. In addition to causing unpleasant smell, organic waste can also cause disease and pollution.

In anaerobic condition, organic waste can decompose into CO_2 gas and CH_4 gas. These gases are categorized as gases that can damage the ozone layer (Rachmawati and Herumurti, 2015). Further, the destruction of ozone layer causes the ultraviolet rays, which are free radicals, to be able to expose earth directly.

Various kind of innovations to prevent free radicals are widely practiced. One of them uses a compound known as antioxidant. Some examples of antioxidant compound are phenolic acids, anthocyanins, and ascorbic acid, which are are often found in functional food (Xu, 2012). In addition to these compound, there are other compounds that function as antioxidant, one of them is ß-glucan, which is not widely known by the public. ß-glucan has several advantages such

as the ability to easily absorb by the body (El Khoury *et al*., 2012). Furthermore, other biological activity of ß-glucan is that it is able to act as antioxidant, antitumor, anti cholesterol, and potentially increase the immune system (Bashir and Choi, 2017; Rahar *et al*., 2011; Vetvicka *et al*., 2019).

One source of ß-glucan is the cell wall of microorganism. One of microorganisms that have the potential strains producing ß glucan is *Saccharomyces cerevisiae* which is non – pathogenic and non – toxic, therefore these microorganisms are often used for fermentation of food products(Pengkumsri *et al*., 2017). ß-glucan and *S. cerevisiae* are categorized as *Generally Recognized As Safe* (GRAS) by the FDA (*Food and Drug Administration*), meaning that it has no toxicity or side effects (Leentjens *et al*., 2014). ß-glucan contained in the *S. cerevisiae*'s cell wall and has the potential also safe to be a food additive (Aimanianda *et al*., 2009). *S. cerevisiae* cell wall establish 15 to 30% of the dry weight of the cell, then the more population of *S.cerevisiae* grown the more ß-glucan is produced (Lesage and Bussey, 2006).

S. cerevisiae can be cultivated through fermentation with using high carbohydrate substrate. One of the high carbohydrate substrate and not yet widely used is vegetable and fruit waste. In addition to high carbohydrates, the availability of vegetable and fruit waste in Indonesia is very large (Utama *et al*., 2019). Three kinds of vegetable and fruit waste that have the highest amount and from year to year keeps increasing are banana waste, papaya waste, and napa cabbage waste (Central Bureau of Statistics, 2015).

Based on that, the napa cabbage waste, banana waste, and papaya waste can be used as an alternative fermentation medium of *S.cerevisiae* to produce ß-glucan. However, the biological potential of ß-glucan such as antioxidant should be determined. This research was conducted to determine the antioxidant activity of ß-glucan and to know which kind of waste that can be utilized as the best fermentation medium.

MATERIALS AND METHODS

S. cereviseae Growth

Commercial *S. cerevisiae* from Fermipan used in this research. One gram of *S. cerevisiae* was taken then mixed into 9 ml Yeast Glucose (YG) broth and incubated for 72 hours at 27 °C. The cell concentration of *S.cereviseae* were determined every 24 hours by measuring optical density at wavelength 600 nm using a UV-VIS 9200 spectrophotometer (Balia *et al*., 2018).

Preparation of Fermentation Medium

Fermentation medium was made by firstly sorting the napa cabbage, banana, and papaya waste that will be used as the fermentation medium. The sorted wastes were then cutted and blendered. The ratio of waste mixed with the 10% sugar solution is 1:2 (w/v). The semi-solid waste was heated at 75 °C for 15 minutes and cooled until room temperature (27–28 °C). 10% (w/v) of *S. cerevisiae* from Fermipan was inoculated and incubated for 48 hours (Modification of Gunam *et al*., 2011).

Extraction of ß-glucan

The yeast cells were collected via centrifugation of fermentation medium for 10 minutes at 7,500 rpm. The pellets were collected and weighed as mass of cells. Furthermore 15% (w/v) of the cell mass was immersed in pH 5.0 distillation conditioned on a 1.0 M HCl solution and incubated at 50 °C for 48 hours. Cell autolysis was performed by incubating sample for 15 minutes at 80 °C. After incubation, yeast cells were collected by centrifugation for 10 minutes at 5,000 rpm. The obtained pellets were dried at 60 °C until extraction. The pellet was washed with 1.0 M NaOH and mixed for 2 hours at 80 °C. The autolyzed cells were recovered by centrifugation for 25 minutes at 7.500 rpm. The obtained pellet was washed with 1.0 M CH_3COOH and mixed for 2 hours at 80 °C. Pellet is recovered by centrifugation for 25 minutes at 7.500 rpm. The resulting pellet was dried with by freeze drying at – 50 °C and weighed into ß-glucan mass (Pengkumsri, 2016).

Determintation of Antioxidant Activity

ß-glucan and the fermentation medium of each waste are each made into test solutions by extraction using methanol to have a certain concentration as a stock solution. The extraction was done by adding methanol and mixing it for 30 minutes. The mixing results are filtered and fixed on the measuring flask. Each of the test solution was prepared by making series of 2 ml, 1ml, 0.5 ml, 0.25 ml, and 0.125 ml of each test solution and adjusted by methanol to the total volume of 2 ml. Antioxidant activity were determined by adding 0.5 ml of 160 ppm DPPH solution to each series. The series of tests were incubated for 30 minutes and then their absorbance was measured by spectrophotometer at 517 nm wavelength. The obtained absorbance was used to calculate the percentage of inhibition (Mu'nisa, 2012)

$$\% \text{ Inhibition} = \frac{\text{Blank Absobance - Sample Absorbance}}{\text{Blank Absorbance}} \times 100\%. \quad (1)$$

Characterization of β-Glucan Microstructure

ß-glucan was prepared by coating with Palladium Allu and affixed carbon tape to the specimen mount. Then adjusted to the sample surface height and height of the specimen holder surface. Next the sample is tightened with the appropriate coupler. The visualization and photography of the samples were performed using a Jeol JSM – 6360LA Electron microscope at an acceleration voltage of 10 Kv (Novák *et al*., 2012).

RESULTS AND DISCUSSION

The Growth of *S. cereviseae*

S. cerevisiae growth curve can be seen in Fig. 1. This growth curve is obtained based on the optical density. Fermentation time of 0–24 hours showed an increasing graph with absorbance of 0.302 to 0.393. Fermentation time of 24–48 hours, showed a rapid increase and the highest value is obtained at 48 hours with an absorbance value of 0.489. Decreasing growth of *S. cerevisiae* occurred after 48 hours of fermentation, where at 72 hours fermentation the absorbance value decreased to 0.176. This absorbance value represents the number of *S. cerevisiae* cells, the higher absorbance value the higher number of *S. cerevisiae* cells obtained (Salari and Salari, 2017). Fig. 1 showed the fermentation time from 0–24 hours for *S. cerevisiae* which has the lowest growth rate, where this phase is adaptation phase or lag phase where *S. cerevisiae* were still adapting to the growth environment (Vermeersch *et al.*, 2019).

At 24 to 48 hours, there was an increase in growth rate. This phase is called the exponential growth phase or logarithmic phase. In this phase *S. cerevisiae* reached the peak of growth at 48 hours of fermentation medium. The logarithmic phase occured at 48 hours because at that time *S. cerevisiae* has adapted to the medium environment so that the nutrients of the fermentation medium can be used optimally and *S. cerevisiae* can divide quickly and constantly (Castilleja *et al.*, 2017). In addition, at 48 hours of fermentation, *S. cerevisiae* has produced primary metabolites in the form of organic acids which can affect the pH of the medium to be optimal for growth (Stewart, 2017). After 48 hours, *S. cerevisiae* decreased due to entering the death phase. In this phase, there is a reduction in the number of microorganisms due to production of primary metabolites which are toxic to *S. cerevisiae* (Sanchez and Demain, 2008).

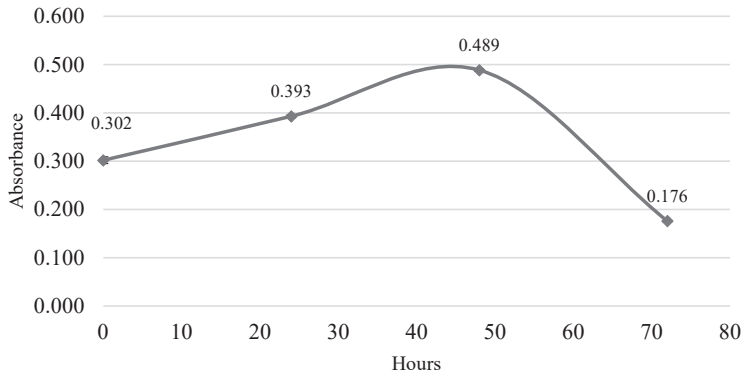
The logarithmic phase is the optimum phase in producing ß-glucan, because at this phase the amount of *S. cerevisiae*'s cells reached its highest, thus the amounts of ß-glucan also reached maximum (Willaert, 2019). Based on Fig. 1, the logarithmic phase occurred at 48 hours of fermentation, therefore this fermentation time was used as the optimal time for the production of ß-glucans from *S. cerevisiae* by using various vegetable and fruit waste as fermenentation medium (banana waste, papaya waste, and napa cabbage waste).

Fermentation medium has an important role in the mass multiplication process of *S. cerevisiae* cells (Mohd Azhar *et al.*, 2017). *S. cerevisiae* requires elements such as C, H, O, N, P, and micro elements such as Fe, Cu, and Mg (Walker and Stewart, 2016). These elements are often obtained in the form of carbohydrates, proteins and minerals. *S. cerevisiae* requires elements of nitrogen obtained from fermentation medium in the form of amino acids and peptides which can be a support in growth metabolism. This nitrogen will be used by *S. cerevisiae* in the process of cell wall formation by affecting the constituent chain of cell walls (Orlean, 2012). The mineral component required for *S. cerevisiae* are magnesium, sodium, calcium, iron, zinc, and others (Eide *et al.*, 2005). These metal ions are used by *S. cerevisiae* to optimize the fermentation process and help the biosynthesis of nucleic acids, phospholipids, and ATP (energy) income (Walker and Stewart, 2016).

The main nutrients in the form of carbohydrates that are often found in the form of sugar are important nutrients in the *S.cerevisiae* fermentation process. Sugar is one of the main component that act as energy producers which found in the form of glucose, sucrose, maltose, cellulose, hemicellulose, lactose, and fructose (Kechkar *et al.*, 2019). The sugar component other than glucose will firstly be converted to glucose in the presence of enzyme, such as invertase and zimase, from *S. cerevisiae* (Ostergaard *et al.*, 2000). Thus, the higher the carbohydrate content, the higher nutrients available thus the higher growth of *S. cerevisiae* (Broach, 2012).

The Production of ß-Glucan with the Utilization of Vegetable and Fruit Wastes

The results of cell mass and the mass of ß-glucan from *S. cerevisiae* cultivated in different fermentation medium such as banana waste, papaya waste, and napa cabbage waste can be



1: *The Growth Curve of S. cerevisiae*

seen in Fig. 2. Based on Fig. 2, it is known that fermentation using banana waste as medium resulted as many as 144.399 g of cell mass and 21.619 grams of ß-glucan mass. Papaya waste fermentation medium produced 139.518 g of cell mass and 19.094 grams of ß-glucan mass, and napa cabbage waste medium produced 74.810 g of cell mass and 9.802 g of ß-glucan mass. The highest amount of *S. cerevisiae* cell mass is produced by using fermentation medium from banana waste. This could be caused by the nutritional content of banana waste, which has the highest carbohydrate content compared to fermentation medium made by papaya waste and napa cabbage waste (Pyar and Peh, 2018).

The mass of *S. cerevisiae* cells from papaya waste as fermentation medium is higher than napa cabbage waste as fermentation medium, but lower than banana waste as fermentation medium. This situation can occur because the number of carbohydrate from papaya waste is lower than banana waste but higher than napa cabbage waste, thus the resulting cell mass yield is lower than the cell mass from banana waste fermentation medium but higher than the amount of cell mas from napa cabbage fermentation medium (Pyar and Peh, 2018; Saran *et al.*, 2016; You *et al.*, 2017).

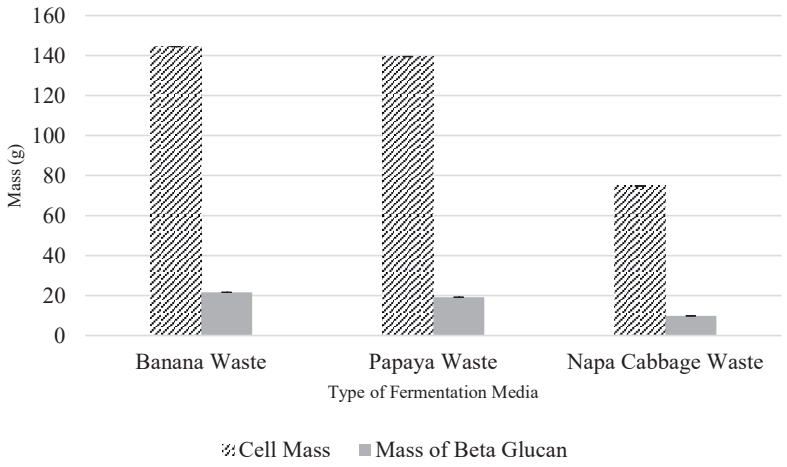
The cell mass was obtained by cell autolysis or breakdown of cells to obtain ß-glucans (Piotrowska and Masek, 2015). The ß-glucan mass will be in line to the mass of the cell produced. This occured because ß-glucan is obtained from the cell wall, the number of cells will determine the amount of ß-glucan produced (Aimanianda *et al.*, 2009). Components that play an important role in the formation of ß-glucan are also glucose. Therefore, the highest ß-glucan mass is produced by using banana waste fermentation medium because the highest glucose content is found in banana waste so that it can maximize the ß-glucan production process (Pengkumsri *et al.*, 2017; Pyar and Peh, 2018). The metabolism of the formation of ß-glucan is in the presence of glucose which is converted to glucose-6-phosphate wherein the presence of the enzyme phosphoglucomutase is obtained by glucose-1-phosphate and is broken down into UDP-Glucose which is the constituent component of the cell wall of *S. cerevisiae* (Orlean, 2012).

The mass of ß-glucan and cell mass can be used to find the percentage of ß-glucan produced based on the cell mass obtained. The highest percentage of ß-glucan produced was obtained from banana waste which was 14.972%, followed by the mass of ß-glucan from the papaya waste as fermentation medium with 13.686%, and the mass of ß-glucan with napa cabbage waste as fermentation medium as much as 13.102%. In general, the percentage of ß-glucan to cell mass obtained from *S. cerevisiae* is 9–11% in standard medium (Piotrowska and Masek, 2015). The results show higher percentage of ß-glucan obtained from fermentation medium with vegetable and fruit waste supplementation.

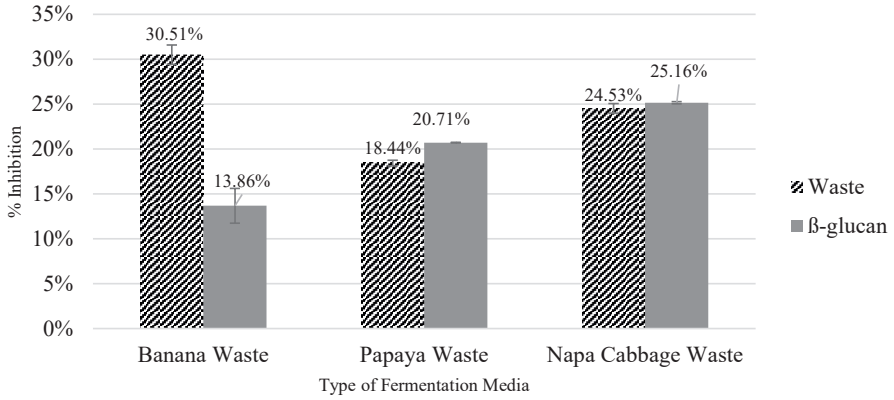
This percentage represents the total percentage of ß-glucans. Details of the percentage of the ß-glucan types from the *S. cerevisiae* cell wall were 50–55% in the form of (1,3) ß-D-glucan, 5–10% in the form of (1,6) ß-D-glucan, and 3–7% in the form of (1,4) α (1,3) ß-D-glucan (Aimanianda *et al.*, 2009; Klis *et al.*, 2002; Ruiz-Herrera and Ortiz-Castellanos, 2019). The dominating type of ß-glucan bond from *S. cerevisiae* is a special feature of the ß-glucan produced. This ß-glucan type (1,3) ß-D-glucan has a higher biological activity compared to other types of ß-glucans (Bashir and Choi, 2017). This is due to the small molecular mass (1,3) ß-D-glucan, where the smaller molecular mass allows higher biological activity is produced (Rahar *et al.*, 2011).

Antioxidant Activity of ß-glucan from *S. cereviseae* with Vegetable and Fruit Wastes as Fermentation Medium

Antioxidant activity of waste and ß-glucan extract can be seen in Fig. 3. Antioxidant activity of banana waste fermentation medium was obtained



2: *β-glucan Production with Vegetable and Fruit Wastes*



3: *Antioxidant Activity from Various Wastes and Extracted ß-glucan*

by 30.51% and the antioxidant activity of ß-glucan using banana waste as fermentation medium was obtained by 13.68%. Fermentation medium of papaya waste was 18.44% while antioxidant activity of ß-glucan from the fermentation medium was 20.71%. The results of antioxidant activity on napa cabbage waste as fermentation medium were 24.53% and 25.16% from ß-glucan produced.

In general, the antioxidant activity of ß-glucan derived from yeast has 10% of inhibition (Kofuji *et al.*, 2012). Based on the results, ß-glucan antioxidant activity obtained higher activity compared to the activity of ß-glucan from yeast in general, this can occur because the ß-glucan obtained is possible not only from *S. cerevisiae* but also with ß-glucan from fermented medium which is extracted (Taurisano *et al.*, 2014).

The results of the comparison of antioxidants from wastes with ß-glucans has different activity in each type of waste. The antioxidant activity of banana waste is higher than the activity of ß-glucan antioxidant extracted by banana waste fermentation medium, but when viewed from the large decrease in antioxidant activity of each concentration, antioxidant activity in banana waste has a high difference. It can be concluded that the antioxidant activity of banana waste is indeed higher but less stable when compared with the antioxidant activity of ß-glucan extracted by the fermentation medium of banana waste, which had a lower antioxidant activity but a more stable decrease. Compounds that play an important role as antioxidants in banana waste are flavonoids, which does have a less stable structure (Bhatt and Patel, 2015; Sidhu and Zafar, 2018). The antioxidant activity of banana waste is also possible from amino acids, peptides, flavonoid compounds, catecholamines, dopamine, dopamine polymers, and it is possible that the ß-glucan component is counted as an antioxidant activity from banana waste (Torres-León *et al.*, 2018; Varzakas *et al.*, 2016).

Antioxidant activity of ß-glucan extraction results was higher than the antioxidant activity of papaya waste. This happened because ß-glucan were extracted so produced higher biological activity (Wang *et al.*, 2017). The main antioxidant component of papaya waste itself is polyphenol (Verghese *et al.*, 2016). Polyphenol has a high sensitivity level so it is possible that it was damaged during process (Gunathilake *et al.*, 2018).

The antioxidant activity of ß-glucan extracted from the medium of napa cabbage waste fermentation has more antioxidant activity compared to the antioxidant activity of the napa cabbage waste itself. This can occur because the antioxidant component of napa cabbage waste is ß-carotene and vitamin C which is less stable, thus resulted in low antioxidant activity (Anwar *et al.*, 2018). It could also due to the ß-glucan found in napa cabbage waste does not work optimally because it was still in crude form. Meanwhile the antioxidant activity of ß-glucan produced by the medium of napa cabbage waste fermentation can produce higher results because the ß-glucan is purer due to the extraction process.

The antioxidant activity of ß-glucan from each fermentation medium also showed different values. This difference in antioxidant activity could occur due to the different characteristics of each ß-glucan extracted. The difference of the components contained in the fermentation medium will affect the fermentation process, especially in terms of the formation of cell wall components, therefore the biological activity of ß-glucan produced are different (Rahar *et al.*, 2011; Varelas *et al.*, 2017). The main components that influence cell wall formation are carbon and nitrogen, which are often found in the form of carbohydrates and proteins (Ogden *et al.*, 2018).

According to (Wu *et al.*, 2008), the best percentage of carbon and nitrogen elements in producing ß-glucan seen from its functional ability is 4% carbon with 2.5% nitrogen which is similar with napa cabbage waste resulting in a higher functional ability compared to banana and papaya waste which both have different content of carbon and nitrogen. In addition, with the highest amount of nitrogen found in napa cabbage waste, the formation of ß-glucan with short branch chains

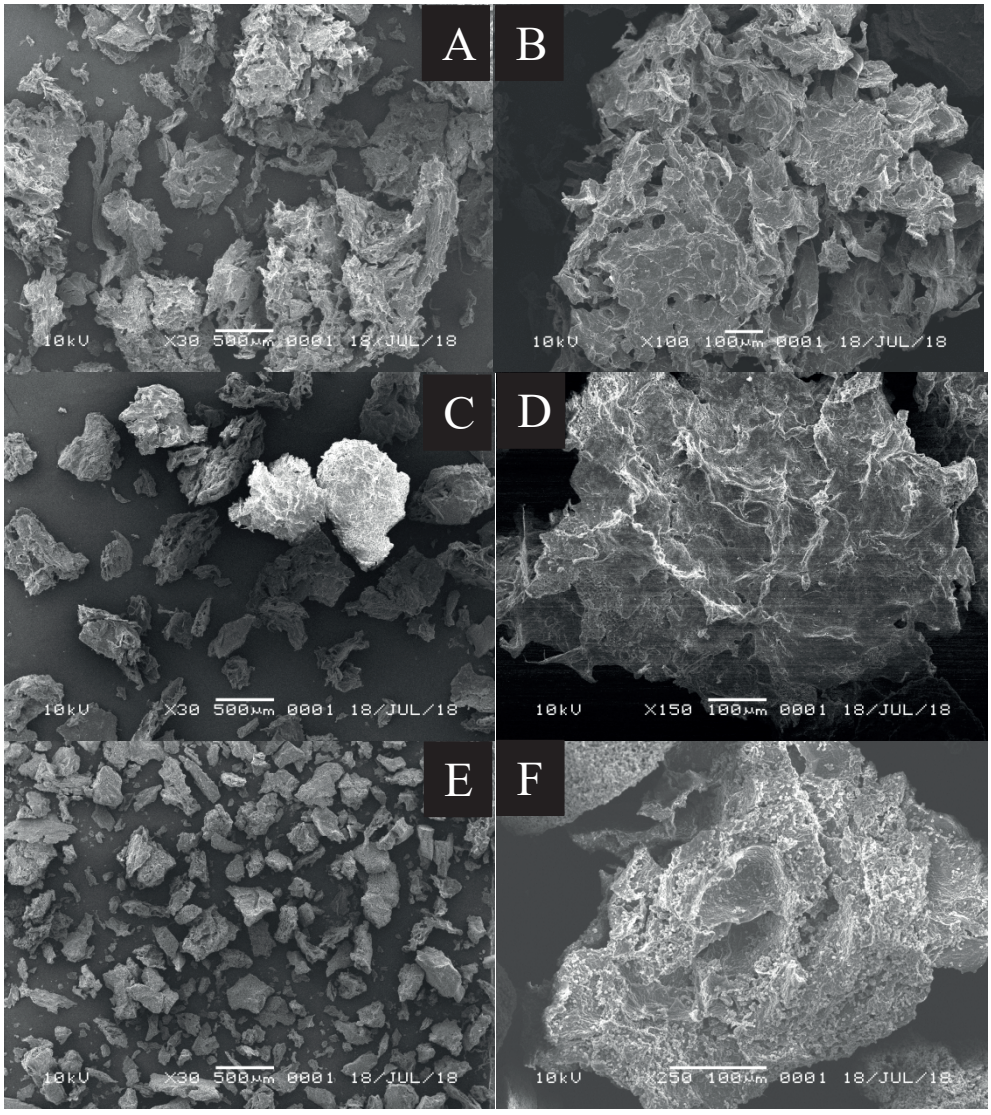
such ad ß- (1,3) -D-glucan and ß- (1,6) -D-glucan, can be more optimum (Aimanianda *et al.,* 2009; Yoshimi *et al.*, 2017). The formation of this short branch chain affects the physical properties of the resulting particle size which will be smaller than the particle size of the long linear chain (Nickels *et al.*, 2016).

The different size of ß-glucan particles will also cause different antioxidant activity of ß-glucan. There is an inverse proportion between antioxidant activity and particle size.The smaller particle size the higher antioxidant activity of ß-glucan (Kurek *et al.*, 2016). The small size of ß-glucan having also a small molecular mass, will enable it to dissolve more readily thus making the antioxidant higher (Wang *et al.*, 2017).

These results are supported by the ß-glucan microstructure which can be seen in Fig. 4.

At 30× magnification, the size of ß-glucan particles from each fermentation medium was obtained at 700µm from the banana waste fermentation medium, 500 µm from the fermentation medium for papaya waste, and 300 µm from the napa cabbage waste fermentation medium, while for globular of ß-glucan obtained different magnifications from each fermentation medium. Globular ß-glucan with banana waste fermentation medium has a diameter of 825 µm measured at 100× magnification. ß-glucan with papaya waste fermentation medium also has a diameter of 533 µm using 150× magnification. ß-glucan with napa cabbage waste fermentation medium obtained globular with 370 µm diameter at 250× magnification.

These results showed that the diameter of ß-glucan from each fermentation medium have different size, while commercial globular ß-glucans



4: *The microstructure of ß-glucan produced by banana waste fermentation medium (A and B), ß-glucan produced by papaya waste fermentation medium (C and D), and ß-glucan produced by napa cabbage fermentation medium (E and F)*

have diameter from 5 µm to 100 µm. This occurred due to differences in the substrate used to produce ß-glucans and its purity (Piotrowska and Masek, 2015). ß-glucan microstructure with fermentation medium of napa cabbage waste has the smallest microstructure size, meaning it has the highest solubility thus resulted in the highest antioxidant activity (Wang *et al.,* 2017). Meanwhile ß-glucan derived from papaya waste medium has a greater size than napa cabbage's but smaller size than banana's. Napa cabbage waste resulting the lower antioxidant activity than ß-glucan with napa cabbage waste fermentation medium but higher than the antioxidant activity of ß-glucan with banana waste fermentation medium. ß-glucan from banana waste fermentation medium has the largest microstructure size and lowest solubility therefore the lowest antioxidant activity produced.

Based on visual observation, each ß-glucan has similar globural shape, but when viewed extensively the shape of each ß-glucan particles has different size. The different carbon and nitrogen content in banana waste, papaya waste, and napa cabbage waste affect the of cell wall's size, especially the formation of ß-glucans (Wu *et al.*, 2008). The higher availability of carbon and nitrogen in the fermentation medium used, the greater ß-glucan's particle size produced (Upadhyay *et al.*, 2017).

Largest ß-glucan particle size is obtained from the fermentation medium of banana waste which has the highest carbon content with the lowest nitrogen content (Pyar and Peh, 2018). Papaya waste containing lower carbon but higher nitrogen than banana waste, so that the size of ß-glucan particles were also smaller than the ß-glucan particles of banana waste (Saran *et al.*, 2016). Meanwhile napa cabbage waste has the lowest carbon but highest nitrogen therefore it produced ß-glucan with the smallest particle size (You *et al.*, 2017).

CONCLUSION

Papaya waste chosen as the best fermentation medium in producing the number of ß-glucan mass (19.094 g) with antioxidant activity of 20.71%. The identification of ß-glucan microstructur shown papaya waste fermentation medium resulted ß-glucan globular diameter of 533 µm.

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