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POTENSI PREBIOTIC DARI EKSTRAK JAMUR TIRAM PUTIH (*PLEUROTUS OSTREATUS*)

[*Prebiotic potency from White Oyster Mushroom (Pleurotus ostreatus) Extract*]

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ABSTRACT

Nowadays, people are more aware of a healthy lifestyle and demand more functional food products. It leads to the raising of prebiotics and their health benefit, such as maintaining digestive tract health, decreasing heart disease and obesity risk, and improving the immune system. Since people need to diversify healthy food product, the pursuit of novel prebiotic ingredient which is potentially incorporated into functional food product needs to be done. One substance that has potency is β-glucan from white oyster mushroom or *Pleurotus ostreatus*. Accordingly, the aim of this study is to observe the prebiotic potency of white oyster mushroom extract. The study started by extracting β-glucan from white oyster mushroom powder by hot water extraction and subsequently proceeded into alkaline extraction. After that, β-glucan content of extracts and residue were measured by Megazyme® β-glucan assay kit and supplemented in glucose-free growth media to see whether it can be utilized by probiotic *Lactobacillus plantarum* Dad-13 and pathogen *Escherichia coli* InaCC B-4. After prebiotic index, prebiotic activity score, pH, and titratable acidity of each extract were compared to FOS and inulin, it showed that β-glucan from water extract of *P. ostreatus* has the potency to become a novel prebiotic substance. It has 37.15+1.27 g/100g β-glucan content, 1.42+0.05 prebiotic index, and 0.91+0.01 prebiotic activity score. It could be utilized by probiotic to produce organic acid, such as lactic acid as well.

Keywords: prebiotic, extraction, β-glucan, *Pleurotus ostreatus*

ABSTRAK

Dewasa ini, kesadaran masyarakat akan gaya hidup yang sehat menyebabkan meningkatnya kebutuhan masyarakat akan produk pangan fungsional. Hal tersebut meningkatkan popularitas prebiotik dengan berbagai manfaat kesehatannya seperti menyehatkan saluran pencernaan, mencegah penyakit jantung dan obesitas, serta meningkatkan imunitas. Seiring dengan meningkatnya permintaan konsumen akan diversifikasi produk pangan sehat, maka perlu dilakukan pengungkapan potensi bahan prebiotik baru yang berpotensi untuk dijadikan produk pangan fungsional. Salah satu bahan yang berpotensi adalah β-glukan dari jamur tiram putih atau *Pleurotus ostreatus*. Maka dari itu, tujuan dari penelitian ini adalah untuk menguji potensi prebiotik dari ekstrak jamur tiram putih. Penelitian ini diawali dengan ekstraksi β-glukan dari tepung tubuh buah jamur tiram putih dengan ekstraksi air panas dan dilanjutkan dengan ekstraksi alkali. Kemudian, masing-masing ekstrak dan residu padatan diukur kandungan β-glukannya dengan Megazyme® *β-glucan assay kit*. Setelah itu, masing-masing ekstrak disuplementasikan kedalam media pertumbuhan *Lactobacillus plantarum* Dad-13 dan *Escherichia coli* InaCC B-4 untuk kemudian diukur nilai indeks prebiotik, skor aktivitas prebiotik, pH media dan asam titrasinya dan dibandingkan dengan FOS dan inulin. Dari hasil penelitian ini diperoleh β-glukan dari ekstrak air *P. ostreatus* berpotensi untuk menjadi bahan prebiotik baru dengan kandungan β-glukan 37,15±1.27 g/100g, 1,42±0.05 indeks prebiotik, dan 0,91±0.01 skor aktivitas prebiotik serta mampu difermentasikan menghasilkan asam-asam organik seperti asam laktat oleh probiotik. β-glukan, *Pleurotus ostreatus*.

Kata Kunci: prebiotik, ekstraksi, β-glukan, *Pleurotus ostreatus*

INTRODUCTION

Prebiotic is defined as "A non-digestible compound that, through its metabolization by microorganisms in the gut, modulates the composition and/or activity of the gut microbiota, thus conferring a beneficial physiologic effect on the host" (Bindels *et al.,* 2015). Some benefits of consuming prebiotic have been mentioned in several studies such as improving beneficial microbiota which inhabit our gut, like lactobacili and bifidobacteria, and decreasing pathogenic microbiota at the same time. These beneficial bacteria produce short-chain fatty acids (SCFA) such as acetate, butyrate, and propionate from prebiotic fermentation. This effect leads to various health benefits, for instance lowering the risk of gastrointestinal disorders including infection, inflammatory bowel disease, and colon cancer. Other health benefit is enhancing several mineral absorptions, improving gut barrier function, developing better immune system defence, lowering risk factors of coronary heart disease and promoting satiety and weight loss, hence preventing obesity (Slavin, 2013; Carlson *et al.,* 2018).

Consumer nowadays has grown their awareness of healthy lifestyle and demand healthy food product to accommodate their healthy diet. It is indicated by the term functional food, food that offers additional health effects besides its nutrition, which is getting more popular than before. With their numerous health benefit, prebiotics have the potency to be alternatively incorporated into a functional food product. Inulin, fructooligosaccharide (FOS) and Galactooligosaccharide (GOS) have been largely observed for their prebiotic ingredients and can be found in various food products (Cardoso *et al.,*

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2021). However, with the high demand for prebiotics and functional food products as well, the search for novel sources of prebiotics that is relatively cheaper and locally available is needed.

To be classified as a prebiotic, some criteria have to be met which are: They cannot be digested by the human digestive tract, they can be fermented by gut microbiota, and they selectively encourage the growth and/or activity of beneficial gut bacteria (Gibson *et al.,* 2017). Edible mushrooms contain polysaccharides that potentially possess prebiotic activity, for instance, chitin, hemicellulose, mannan, α- and β-glucans. Linear and branched glucans linked by various types of glycosidic bonds such as 1–3 or 1–6 – β -glucans are one of the major structures of polysaccharides in edible mushrooms (Wasser, 2003; Jayachandran *et al.,* 2017). Since β-glycosidic bonds cannot be digested by digestive enzymes secreted by the mammalian pancreas (van Loo, 2012), β-glucan in mushrooms has the potency to be distinguished as a prebiotic.

β-glucan can be found in various sources, such as yeast, fungi, bacteria, algae and cereal, and is known to have bioactivity such as hypocholesterolemic, hypoglycemic, immunomodulatory, antitumor, antioxidant and anti-inflammatory. Du *et al.* (2019) reported that there is a correlation between the sources and structure of β-glucan to its biological activity. Although the use of β-glucan from several sources, such as oat and barley, have been observed to have prebiotic properties in vitro and in vivo experiment (Jayachandran *et al.,* 2018), prebiotic properties of mushroom β-glucan is rarely discussed.

Several studies have been conducted to examine the prebiotic properties of mushrooms and found that their unspecified polysaccharide may have the ability to be prebiotic (Nowak *et al.,* 2017; Sawangwan *et al.,* 2018; Li *et al.,* 2019; Asad *et al.,* 2020; Setyawan and Kamil, 2021) and found that different kind of mushroom has different prebiotic property. β-glucan from several kinds of mushrooms as in *Schizophyllum commune* Fr and *Auricularia auricula* Judae (Chaikliang *et al.,* 2015) and *Sparassis* sp. (Jeong *et al.,* 2017) proved to have prebiotic properties. However, the diverse structure and physicochemical properties of β-glucan in mushrooms result in different prebiotic activity as well (Ruthes *et al.,* 2021). Nonetheless, β-glucan from white oyster mushroom (*P. ostreatus*) has not been well observed to have prebiotic properties, despite its potency to be incorporated in functional food (Saskiawan *et al.,* 2017, 2018; Setyawan *et al.,* 2021) and has proven to promote the growth of certain lactic acid bacteria (Kokina *et al.,* 2018). Therefore, the purpose of this research is to extract β-glucan from white oyster mushrooms and observe its prebiotic properties as a novel source of prebiotic.

Material

The white oyster mushroom was collected from the collection of the Research Center for Applied Microbiology, National Research and Innovation Agency. *Lactobacillus plantarum* Dad-13 was obtained from Food and Nutrition Culture Collection (FNCC) and possesses probiotic activity (Rahayu *et al.,* 2016, 2019, 2021), while pathogen *Escherichia coli* InaCC B-4 was obtained from Indonesia Culture Collection (InaCC). Both bacteria were kept in 0.2 mL glycerol 20% and skim milk 10% with a 1:1 ratio and froze at -80°C.

The media were deMan Ragosa Sharpay (MRS) broth (Himedia®), Tripton Soy (TS) broth (Oxoid® and bacterial agar. Glucose-free MRS broth was made by dissolving peptone from casein (10 g/L), meat extract (8 g/L), yeast extract (4 g/L), di-potassium hydrogen phosphate trihydrate (2 g/L), tween 80 (1 g/L), ammonium citrate tribasic (2 g/L), sodium acetate (5 g/L), magnesium sulfate heptahydrate (0.2 g/L) and mangan sulfate tetrahydrate (0.04 g/L). Glucose-free TS broth was made by dissolving tryptone (17 g/L), peptone from enzymatically digested soybean (3 g/L), sodium chloride (5 g/L), di-potassium hydrogen phosphate trihydrate (2.5 g/L). D-glucose was used as the positive control (20 g/L for MRS broth and 2.5 g/L for TS broth), while FOS from chicory (Sigma®) and inulin from chicory (Sigma®) were used as commercial prebiotic control. All materials were purchased from Merck® as an analytical grade unless otherwise stated.

Mushroom extraction

The harvested fruiting body of the white oyster mushroom was cleaned and sliced into pieces before it was steamed over boiling water for 10 minutes for blanching. After that, it was dried in an oven dryer at 60°C for 24 hours, then blended and sieved through a 40 mesh sieve. Finally, the collected mushroom flour was kept in an air-tight container.

Mushroom flour was firstly extracted using distilled water at 90°C for 4 hours with a ratio of flour: water = 1: 20. Then, the water-soluble extract (WE) was separated by centrifugation at 9000 G for 15 minutes and washed twice. The obtained pellet was then proceeded to the alkaline extraction stage using 1 M NaOH solution at 4° C for 4 hours with the volume of the solution equal to the volume of water. The alkaline was then separated through centrifugation and the alkaline-soluble extract was acquired (AE). Extracts from each extraction were then concentrated using a rotary vacuum evaporator to a 4-fold concentration. The AE was then neutralized using glacial CH_3COOH. Finally, both

WE and AE extracts were freeze-dried, while the solid residue of the extraction (S) was dried in an oven dryer at 60°C, for 24 hours. All obtained fractions were stored in tight containers (Synytsya *et al.,* 2009).

β-glucan content measurement

β-glucan content from each extract (WE, AE and S) were analyzed according to β-Glucan Assay Kit (Yeast and Mushroom) (Megazyme®) and calculated by Mega-Calc™ (Megazyme®).

Bacteria preparation

The frozen culture of *L. plantarum* Dad-13 was firstly thawed at room temperature then inoculated into 50 mL of MRS broth media and incubated in a 37°C incubator for 24 hours. Meanwhile, thawed *E. coli* InaCC B-4 was inoculated into 50 mL of TS broth media and incubated in a 37°C incubator for 24 hours.

Prebiotic index and prebiotic activity measurement

The prebiotic index was analyzed to see the ability of each extract to be used as carbon source (2% w/v) by probiotic *L. plantarum* Dad-13 in glucose-free MRS broth. The enriched culture was inoculated with an inoculum size was 1% (v/v) to broth media, then fulfilled to a 15 mL conical tube. FOS and inulin were also used as commercial prebiotic control., while glucose was used as the control carbohydrate After being incubated for 24 hours at 37°C, probiotic colonies were enumerated by serial dilution and plating on MRS agar. The prebiotic index was calculated to see the ability of each extract to be used as carbon sources by probiotics through formula (1).

$$Prebiotic\ index = \frac{(Log\ P24 - Log\ P0)\ extract}{(Log\ P24 - Log\ P0)\ glucose} \quad 1$$

Log P24 is the number of probiotic bacteria after 24-hour incubation. Log P0 is the number of probiotic bacteria before incubation. (Figueroa-González *et al.,* 2019).

The prebiotic activity was analyzed to measure the selectivity of each extract to be used by the probiotic, not by the pathogen. Enriched probiotic *L. plantarum* Dad-13 was inoculated to MRS and extract supplemented MRS broth with 1% (v/v) inoculum size to be fulfilled to a 15 mL conical tube. Then incubated in a 37°C incubator Samples were taken before and after 24-hour incubation and enumerated by serial dilution and plating on MRS agar. A similar method was done for pathogen bacteria *E. coli* InaCC B-4. The differences are pathogen bacteria were inoculated to extracts supplemented with glucose-free TS broth (0.25% w/v) and samples from before and after incubation was enumerated on TS agar.

$$Prebiotic\ activity = \left(\frac{(LogP24 - Log\ P0)\ extract}{(Log\ P24 - Log\ P0)\ glucose}\right) - \left(\frac{(LogE24 - LogE0)\ extract}{(Log\ E24 - Log\ E0)\ glucose}\right) \quad 2$$

Log P24 is the number of probiotic bacteria after 24-hour incubation. Log P0 is the number of probiotic bacteria before incubation. Meanwhile, Log E24 is the number of pathogen bacteria after 24-hour incubation, and Log E0 is the number of pathogen bacteria before incubation (Huebner *et al.,* 2007).

pH and titratable acidity measurement

pH and titrate acid were measured from MRS and extract-supplemented MRS media to detect the acid produced during incubation. The remain of sampled inoculated MRS and extract-supplemented MRS media were centrifuged at 1500 rpm for 15 minutes. Then, the obtained cell-free media proceeded to pH and titrable acidity measurement. the pH of cell-free media was measured by LAQUA PH1100 pH meter (HORIBA®). While the titratable acidity value was measured by 0.1 N NaOH titration with Phenolphthalein 1% as an indicator. (AOAC, 1990).

RESULT

β-glucan content

Extraction by hot water could obtain higher content of β-glucan compared to alkaline with 37.15 $\pm$ 1.27 g/100 g extract to 2.44 $\pm$ 1.21 g/100 g extract (Table 1). However, the solid residue of extraction still contained 32.06 $\pm$3.45 g/100 g extract, which represented the unextracted β-glucan in the sample from water and alkaline extraction.

Table 1. β-glucan content of each extract of white oyster mushroom (*P. ostreatus*) (*Kandungan β-glucan tiap ekstrak jamur tiram putih (P. ostreatus)*).

Extract	β-glucan (g/100 g)
WE	37.15±1.27[a]
AE	2.44±1.21[b]
S	32.06±3.45[c]

*Values represent averages ± standard deviation for duplicate experiments. (*Nilai mewakili rata-rata ± standar deviasi untuk eksperimen duplikat*)

**Values followed by the same letters in each column are not significantly different at the 0.05 level after being statistically tested by one -way ANOVA in SPSS 26.0. (*Nilai yang diikuti huruf yang sama pada setiap kolom tidak berbeda nyata pada taraf 0,05 setelah diuji secara statistik dengan one way ANOVA pada SPSS 26.0.*).

Prebiotic index and prebiotic activity score

The highest prebiotic index was obtained by water extract (1.42±0.05), followed by solid residue and alkaline extract (0.75-±0.06 and 0.41±0.03 respectively) (Table 2). This result was matched with β-glucan content in each extract as shown in Table 1. Among all extracts, water extract was the only extract that had a prebiotic index score higher than 1. Higher than 1 prebiotic index indicates that the substance has higher probiotic growth promotion compared to a positive control (glucose in this experiment) (Palframan *et al.,* 2003). Compared to commercial control of prebiotics (FOS and Inulin), water extract was the only extract that has a higher prebiotic index than inulin and is equal to FOS. It means that the water extract of *P. ostreatus* might promote the growth of probiotic *L. plantarum* Dad-13 better than the other extracts and inulin.

Table 2. Prebiotic index and prebiotic activity score of *Pleurotus ostreatus* extracts in glucose-free MRS medium by *L. plantarum* Dad-13 and glucose-free TS medium *E. coli* InaCC B-4. (*Indeks prebiotik dan skor aktivitas prebiotik ekstrak Pleurotus ostreatus pada medium MRS bebas glukosa oleh L. plantarum Dad-13 dan medium TS bebas glukosa E. coli InaCC B-4*).

Supplement	Prebiotic Index	Prebiotic activity score
WE	1.42 ± 0.05[a]	0.91 ± 0.01[a]
AE	0.41 ± 0.03[b]	-0.52 ± 0.12[b]
S	0.75 ± 0.06[c]	-0.17 ± 0.02[c]
FOS	1.21 ± 0.06[ad]	1.02 ± 0.01[a]
Inulin	1.12 ± 0.15[d]	0.48 ± 0.01[e]

*Values represent averages ± standard deviation for duplicate experiments. (*Nilai mewakili rata-rata ± standar deviasi untuk eksperimen duplikat*)

**Values followed by the same letters in each column are not significantly different at the 0.05 level after being statistically tested by one -way ANOVA in SPSS 26.0. (*Nilai yang diikuti huruf yang sama pada setiap kolom tidak berbeda nyata pada taraf 0,05 setelah diuji secara statistik dengan one way ANOVA pada SPSS 26.0.*).

pH and titratable acidity value

A significant decrease in pH values and increasing titratable acid values in media supplemented by WE, FOS, and inulin after the fermentation of probiotic *L. plantarum* Dad-13 (Table 3). However, there was no significant difference in media supplemented by AE and S, even though there was a decrease in their pH media after the fermentation. The decline of media pH was the result of carbohydrate fermentation by probiotic and produces SCFA and organic acid, such as lactic acid (Gibson *et al.,* 2017). Lactic acid is the main product of carbohydrate fermentation of *L. plantarum,* followed by various acids such as acetic acid, propionic acid, phenyl lactic acid, formic acid and succinic acid (Behera *et al.,* 2018). Hence there was only small activity in the fermentation of *L. plantarum* in AE and S-supplemented media.

Table 3. pH and a titratable acid value of *Pleurotus ostreatus* extracts in glucose-free MRS medium inoculated by *L. plantarum* Dad-13 (*pH dan nilai asam titrasi ekstrak Pleurotus ostreatus dalam medium MRS bebas glukosa yang diinokulasi L. plantarum Dad-13*).

Supplement	pH values		Titratable acid (%)	
	0 H	24 H	0 H	24 H
WE	5.22 ± 0.03^{a}	4.11 ± 0.03^{b}	2.85 ± 0.46^{a}	4.69 ± 0.28^{b}
AE	6.96 ± 0.01^{a}	6.53 ± 0.02^{b}	2.07 ± 0.05^{a}	2.11 ± 0.09^{a}
S	6.49 ± 0.01^{a}	5.71 ± 0.01^{b}	2.3 ± 0.28^{a}	2.3 ± 0.28^{a}
FOS	5.11 ± 0.01^{a}	4.99 ± 0.01^{b}	2.39 ± 0.37^{a}	3.03 ± 0.28^{b}
Inulin	5.14 ± 0.01^{a}	5 ± 0.03^{b}	2.02 ± 0.18^{a}	3.68 ± 0.37^{b}

*Values represent averages ± standard deviation for duplicate experiments. (*Nilai mewakili rata-rata ± standar deviasi untuk eksperimen duplikat*)
**Values followed by the same letters in each column are not significantly different at the 0.05 level after being statistically tested by one -way ANOVA in SPSS 26.0. (*Nilai yang diikuti huruf yang sama pada setiap kolom tidak berbeda nyata pada taraf 0,05 setelah diuji secara statistik dengan one way ANOVA pada SPSS 26.0.*).

DISCUSSION

The difference in β-glucan content extracted from the sample might happen because different solvents may extract different kinds of β-glucan. Higher pH conditions can extract highly branched triple helix β-glucan (Nitschke *et al.,* 2011). In addition, low temperatures in alkaline extraction may affect the yield of β-glucan extraction (Synytsya *et al.,* 2009). This result is also quite different from (Baeva *et al.,* 2019) who reported that they acquired 59.9 % β-glucan from the dry matter of hot water extracted *P. ostreatus*. They also measure the β-glucan of dried alkaline extract and a solid residue of *P. ostreatus* (55.21% and 45.49% respectively). The difference is they performed an anionic exchange and size exclusion preparative chromatography to separate and purify their extracts. Khan *et al.* (2017) reported that 14.182 g/100g of β-glucan was possessed by *P. ostreatus* through hot water extraction. However, they used cold water extraction pretreatment before the hot water extraction. They also did freeze-thawing and dialysis to purify the earned extract. While Vetvicka et. al obtained 2.72 g/100g lyophilized extract by autoclaving (121˚C, 30 minutes) *P. ostreatus* powdered fruiting body (Vetvicka *et al.,* 2019). Nitshke *et al.*'s experiment (2011) showed the content of β-glucan of *P. ostreatus* which was subsequently extracted by alkaline potassium hydroxide and sodium hydroxide (2.2 and 6.01 g/100g respectively).

Despite the fact that β-glucan in the solid residue was significantly lower than water-soluble extract, the solid residue of *P. ostreatus* extraction contained relatively high β-glucan content. It suggests that there is broad potency for extraction method modification to improve the yield of β-glucan. One way to enhance the yield is through microwave-assisting extraction. Frioui *et al.* (2018) reported that higher β-glucan content can be extracted by water from the fruiting body of *P. ostreatus* through a microwave oven for 30 minutes. They claimed that 31.2% β-glucan content extract can be earned by that method, while the conventional boiling water method can only produce 7.9% β-glucan content extract.

FOS and inulin are linear D-fructose polymers linked by β-2,1-glycosidic bonds, with a terminal glucose or fructose component. This bond makes both of them are enabled to be digested by the human digestive system, yet can be utilized by several intestinal commensal microorganisms such as *Bifidobacterium.* Though, β-fructokinase and fructose transporting protein are produced by *L. plantarum*, making them capable to utilize FOS and Inulin as well (Buntin *et al.,* 2017). Mushroom β-glucan contain β-1,3 and β-1,6 glycosidic linkages that may be digested by specific glycoside hydrolases (GH). Enzymes *Bu*GH16, *Bu*GH16 and *Bu*GH158 can split linear endo/exo-β-1,3 linkage. Meanwhile, β-1,6 linkages in mushroom β-glucan can be digested by endolytic *Bu*GH30 (Singh *et al.,* 2020). *L. plantarum* also produces β-glycosidase, hence they are able to utilize β-glucan as well. (Russo *et al.,* 2012)

Setiarto *et al.* (2018) reported prebiotic index score of modified taro flour who is lower than control inulin by using *L. plantarum* D-240. On the other hand, (Lestari *et al.,* 2013) reported a higher prebiotic index of sweet potato fiber extract than FOS and inulin by *L. plantarum* Mut-7. It suggests that different strain of bacteria has different

compatibility with prebiotics, which is confirmed in (Figueroa-González *et al.,* 2019).

Furthermore, water extract was also the only *P. ostreatus* extract that had a positive value of prebiotic activity score (0.92±0.01). It was similar to commercial control FOS (1.02±0.01), but higher than inulin (0.48±0.01). The prebiotic activity score represented the availability of extract to be digested specifically by probiotic *L. plantarum* Dad-13 but not by pathogen *E. coli* InaCC B-4. The negative prebiotic activity of alkaline extract and solid residue (-0.52±0.12, and -0.17±0.02) showed that those extracts can be utilized by probiotics and pathogens as well (Huebner *et al.,* 2007). Therefore the alkaline extract and solid residue of *P. ostreatus* had no potency to be claimed as prebiotic, while the water extract had a potency of prebiotic better than inulin and as good as FOS.

Prebiotic potent of *P. ostreatus* extract with probiotic *L. plantarum* Dad-13 may be combined to create a synbiotic. In order to deliver their benefit, probiotics must resist the human upper digestive tract, reach the intestinal, and colonize. While extreme conditions provided by the human upper digestive tract such as high acidity in the stomach, bile salt, and enzyme stress, β-glucan may provide a protective agent to probiotics and increase its success in reaching the colon (Pia Arena *et al.,* 2017). By creating a synbiotic, the health benefit of each probiotic and prebiotic may be enhanced. This study suggests the possible compatibility of water-soluble β-glucan from *P. ostreatus* with probiotic *L. plantarum* Dad-13 due to its ability to improve probiotic growth. However, further research may be conducted to prove that β-glucan from *P. ostereatus* may increase probiotic *L. plantarum* survival in the human gastrointestinal tract.

Water extract of *P. ostreatus* in this study had a higher prebiotic activity score compared to *Pleurotus sajor-caju* water extract in Mallik and Bhawsar study (2018). It showed a 0.20±0.03 prebiotic activity score when it was tested using *Lactobacillus acidophilus* NCIM 2660 and *E. coli.* Moreover, prebiotic activity score from *P. ostreatus* water extract in this study was higher than the prebiotic activity score from laminaran, a β-glucan from brown algae (*Sargassum crassifolium*). The score was 0.96 when it was fermented by *L. plantarum* 0051 FNCC and *E. coli* 0091 FNCC. However, this score is relatively low compared to inulin used as a control in this study, which is 4.78 (Chamidah *et al.,* 2016).

In the other study, supplementation sweet potato fiber extract demonstrated a greater prebiotic activity score compared to FOS and inulin by *L. plantarum* Mut-7 and *E. coli.* On a contrary, Modified taro flour showed a lower prebiotic activity score compared to inulin control when it was supplemented in *L. plantarum* and *E. coli* growth media in Setiarto *et. al*'s experiment (2018).

The decline of media pH is the result of carbohydrate fermentation by probiotic *L. plantarum* and produces SCFA and organic acid, such as lactic acid (Gibson *et al.,* 2017). Lactic acid is the main product of the carbohydrate fermentation of *L. plantarum,* followed by various acids such as acetic acid, propionic acid, phenyl lactic acid, formic acid, and succinic acid (Behera *et al.,* 2018). A similar result was also presented in Kokina *et al*'s experiment. Supplementation of water extracted β-glucan from *P. ostreatus* in certain lactic acid bacteria inoculated milk has shown decreasing final pH compared to control. It indicates that β-glucan supplementation may result in greater glycolytic activity by lactic acid bacteria (Kokina *et al.,* 2018).

However, there are some limitations of this study such as the absence of a purification method in the extracted samples. The presence of impurities such as phenolic compounds may affect the prebiotic activity assay, as polyphenols are contained by *P. ostreatus* and may be extracted in an aqueous solvent. Polyphenols such as catechins, anthocyanins and proanthocyanidins has been demonstrate their prebiotic activity in preclinical studies (González-Palma *et al.,* 2016; Alves-Santos *et al.,* 2020). In addition, other polysaccharides such as chitin, α-glucan, and galactomannan may also be present in the extract *P. ostreatus* due to lack of purification (Synytsya *et al.,* 2009). Another limitation of this study is in vitro prebiotic assessment may not represent the condition in the animal or human gastrointestinal tract, as it is habituated by millions of complex microbiota (Bajury *et al.,* 2017).

CONCLUSION

Extract of *P. ostreatus* has obtained by hot water extraction of white oyster mushroom fruiting body with β-glucan content 37.15±1.27 g/100g, while alkaline extract only contains 2.44±1.21 g/100g and there is still 32.06±3.45 g/100g in solid residue. Furthermore, water extract is the only extract that has a higher than 1 prebiotic index and positive prebiotic activity score, with prebiotic index of 1.42±0.05 and prebiotic activity score of 0.91±0.01, which is equal to prebiotic control FOS and greater than inulin. All extracts also can be fermented by probiotic *L. plantarum* Dad-13 indicated by decreasing pH and increasing titratable acid in growth media. Therefore, β-glucan in water extract of *P. ostreatus* has the potency to be a novel prebiotic substance.

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CONTRIBUTORSHIP

RHS: Designing experiments, collecting and analyzing data, and writing the manuscript. IW and NW: Supervising whole research. K and M: Assisting in data collection. RHS is the main contributor to this manuscript.

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