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The Potential of Oil Palm Mesocarp Fiber Waste as a Prebiotic Material – Chemical and Microbial Evaluation Using Probiotic *Saccharomyces cerevisiae, Lactobacillus casei* and *Escherichia coli*

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ABSTRACT

Oil palm mesocarp fiber (OPMF), a biomass waste generated during the production of palm oil is rich in polysaccharides that can be converted to value-added product. The potential of cellulose from OPMF as a prebiotic represents an innovative exploration of biomass waste, which has never been undertaken. This study aims to investigate the effect of supplementation of OPMF in the medium on the growth of probiotic *Saccharomyces cerevisiae, Lactobacillus casei* and enteropathogenic *E. coli*, and to ascertain the potential OPMF as prebiotic by quantifying prebiotic activity score (PAS). The research was designed using a Randomized Complete Block Design with a single factor and three replications. The factor was the concentration of OPMF extract added to the growth medium, with seven treatment levels: P0 as control (no addition), P1 (1% glucose), P2 (2% prebiotic inulin), P3 (2% OPMF extract), P4 (4% OPMF extract), P5 (6% OPMF extract), P6 (8% OPMF extract), and P7 (10% OPMF extract) (w/v). The results showed that supplementation of OPMF extract significantly supported the growth of both of probiotics used in this study ($p < 0.05$) similar to on the prebiotic inulin. The growth of *S. cerevisiae* was the highest on the 8% OPMF extract, with the PAS value of 1.90. In addition, the growth of *L. casei* on OPMF extract at the minimal concentration of 6% and on inulin were higher significantly than on glucose, with the PAS in the range of 1.98–2.47. In contrast, the growth of *E. coli* on the OPMF extract and on inulin were lower than on glucose ($p < 0.05$). Conclusion, the growth of *L. casei* on OPMF extract was higher than *S. cerevisiae*, at a minimal concentration of 6%. Therefore, OPMF extract was potential as prebiotic.

Keywords: acid hydrolysis, OPMF extract, prebiotic activity score, *S. cerevisiae*, *L. casei*.

INTRODUCTION

During palm oil (*Elaeis guineensis* Jacq) production, solid and liquid biomass waste will be produced. Oil palm mesocarp fiber one of the solid wastes from palm oil production reaches 13% of total production in Indonesia [Ghartina et al., 2019]. Traditionally, OPMF is used in mulching or boiler fuel [Hau et al., 2022; Supriatna et al., 2022; Hartanto and Ratnawati, 2010] which may be detrimental to the environment. Consequently, an alternative biotechnology procedure for overcoming the problem has been required. Some researchers generally use mesocarp in non-food and non-feed fields, such as the use of 10% mesocarp fibre as an alternate for synthetic fibres in making fiberglass composites [Sembiring et al., 2023]; hydrothermal-production of biodegradable nanocellulose from OPMF [Bakar et al., 2021); cellulose microfibers as probiotic encapsulants for *L. fermentum* with a decrease in viability of less than 0.5 log CFU/mL after 35 days of cold storage [Pato et al., 2021]. Similar research was conducted by Chen et al. (2015) reported that prebiotic oligosaccharides from palm kernel peel significantly supported the growth of

Lactobacillus and *Bifidobacterium* and reduced the growth of *E. coli*. Accordingly, the potential of cellulose from OPMF as a prebiotic represents an innovative exploration of biomass which has never been undertaken.

Generally, OPMF, comprises of cellulose, hemicellulose and lignin polymers [Corley and Tinker, 2015]. OPMF is rich with various carbohydrate sugars and other useful chemical compounds that can be converted into a range of value-added products. OPMF contained of cellulose of about 42.7–65%, and hemicellulose of about 17.1–33.5% lignin (13.2–25.31%), moisture (11.10%), ash (1.3–7.9%), glucose (66.4%) [Juliantoni et al., 2018; Sundalian et al., 2021]. Cellulose $(C_6H_{10}O_5)_n$ is a major carbohydrate component found in plant cell walls. It is a hydrophilic polymer with three OH groups per glucose unit, linked by β-1,4-glycosidic bonds. As an insoluble dietary fibre, cellulose resists digestion and absorption in the human body, contrasting with soluble fibres such as pectin, gum, and beta-glucan [Dhingra et al., 2012].

In plant tissues, cellulose is trapped within hemicellulose and lignin, therefore, lignin must be removed to get cellulose. Lignin is a constituent of plant wood tissue composed of phenolic polymers which are strongly bound to cellulose and hemicellulose to form a rigid cell wall structure [Al-Rajabia and Haan, 2021]. Alkaline pretreatment has been identified as one of the best chemical pretreatment methods for delignification of lignocellulosic biomass [Chin et al., 2013]. Hemicellulose is an alkaline soluble carbohydrate while cellulose is acid soluble carbohydrate [Hau et al., 2022]. In this study, acid hydrolysis using H_2SO_4 was employed following sodium hydroxide pretreatment [Nazir et al., 2013; Raharja et al., 2004]. Acid hydrolysis breaks down polysaccharide chains in the fibre, enhancing the solubility of the resulting cellulose.

Prebiotics are undigested carbohydrate in the digestive enzymes but able to stimulate the growth of beneficial gut bacteria, and inhibit the growth of entomopathogens [Gibson and Roberfroid, 1995]. Lactic acid bacteria and Bifidobacteria are the well-known probiotics, and recent findings have reported that *Saccharomyces cerevisiae* var. *boulardii*, *Kluyveromyces marxianus*, and *Pichia kudriavzevii* also possess probiotic properties [Metzler et al., 2005]. In the body, prebiotics promote the growth of probiotics or beneficial microorganisms, thereby contributing to overall health [Kusmiyati, 2020]. According to Roberfroid [2007], foods containing oligosaccharides or polysaccharides (including dietary fibres) have the potential to exhibit prebiotic activity. Dietary fibers are categorized into two types: water-insoluble fibers and water-soluble fibers. Sources of water-soluble fibers including gums, pectin, beta-glucans, and certain soluble hemicelluloses are found in plant cell walls; while, water-insoluble fiber sources include cellulose, lignin, hemicellulose, and chitin [Sulistijani, 2001]. It was suggested that cellulose from OPMF may be potential as a prebiotic derived from non-starch polysaccharides (NSPs) [Aryati et al., 2020]. The potential of a substance as a prebiotic is determined by quantitative measurement of the prebiotic index in estimating the ability of the prebiotic to support probiotic growth but not enteric bacteria [González et al., 2019]. In this research, the ability of OPMF to support the growth of probiotic *S. cerevisiae* and *Lactobacillus casei* demonstrate the prebiotic potential OPMF. Therefore, the objective of the research was to measure the increase growth of *S. cerevisiae* and *L. casei* as probiotics and *E. coli* to ferment OPMF at different levels, and to quantify the prebiotic activity score for the growth of probiotics in media containing OPMF.

MATERIALS AND METHODS

This research was conducted in the Agricultural Product Processing Laboratory, Agroindustry Waste Processing Laboratory, Agricultural Microbiology Laboratory, Department of Agricultural Product Technology, and Biotechnology Laboratory, Department of Plant Protection, Faculty of Agriculture, University of Lampung, from November 2023 to January 2024.

Materials

Materials used in this study include OPMF from PTPN VII (Lampung, Indonesia), *Saccharomyces cerevisiae* and *Lactobacillus casei* were procured from Biotechnology Lab, Universitas Gadjah Mada-Yogyakarta, PDA (potato dextrose agar), NB (Nutrient Broth), NA (Nutrient Agar), EMBA (eosin methylene blue agar), MEA (malt extract agar), MEB (malt extract broth), pathogenic *Escherichia coli* was procured from Balitvet-Lampung, filter paper, 96% ethanol, 2M NaOH, distilled water, inulin, NaCl, H_2SO_4, Na_2CO_3, and alcohol.

Research method

The research consisted of preparation of OPMF flour and assessment of its prebiotic potential for probiotic and enterobacterial growth. The research was employed a randomized complete block design (RCBD) with a single factor. The single factor was concentration of OPMF extract (P) added to the growth medium, with seven levels: P0 (growth medium), P1 (growth medium + 1% b/v glucose), P2 (growth medium + 2% inulin), P3 (growth medium + 2% OPMF extract), P4 (growth medium + 4% OPMF extract), P5 (growth medium + 6% OPMF extract), P6 (growth medium + 8% OPMF extract), and P7 (growth medium + 10% OPMF extract). The experiment was performed in three replications. Data were analysed using Analysis of Variance (ANOVA) followed by the least significant difference (LSD) test at a 5% significance level and assessed using SPSS tools.

Extraction of oil palm mesocarp fiber

The extraction of OPMF was started with the preparation of OPMF flour following the procedure of Bakar [2009]. 500 grams of OPMF were washed, drained for about 30 min, cut into 5 cm pieces, and oven-dried at 105 °C for 3 h. The dried OPMF was then grounded to 60 mesh. The yield of mesocarp flour was 400 grams. The extraction of OPMF flour followed the method described by Raharja et al. [2004] including delignification, bleaching, and acid hydrolysis. Delignification was done by heating OPMF flour in 10% w/v NaOH (2 M) at 80–85 °C for 1 hour. After that, it was sieved and washed with water to neutralize the pH from 9.0 to 6.5–7.0, and then was sun dried. The next step was bleaching carried out by heating the material in a 10% b/v H_2O_2 at 85–90 °C for 1.5 hours. After bleaching, the flour was repeatedly washed with distilled water until neutral pH was achieved (from 3.0 to 6.5–7), and then it was sun dried again. Following step was hydrolysis done by heating the dried material in a 2% H_2SO_4 at 115 °C for 2 hours. Following hydrolysis, the materials was separated and neutralized through repeated washing with distilled water to obtain the cellulose OPMF extract, which was then dried and stored in an airtight container at -5 °C until further use.

Activation of *L. casei*, *S. cerevisiae* and *E. coli*

Saccharomyces cerevisiae, Lactobacillus casei and E. coli were activated before used in experiment. The activation of *L. casei* was run by transferring one loop needle in to a test tube containing 7 mL MRS broth, stirred with vortex and incubated at 37 °C for 24 h. A cloudy appearance of the broth media in the test tube indicated an active culture. Similar to *L. casei, S. cerevisiae* and *E. coli* were inoculated in to ME broth, and Nutrient broth respectively. When all the cultures were active by showing cloudy appearance on the test tube, they were kept in refrigerator until used.

Mirobial strains and prebiotic activity assay

The growing cultures were straked on the agar plate and incubated, according to designed temperature and time, at 27 °C for 24h, 42 °C for 48h, and 32 °C for 24 h for *S. cerevisiae, L. casei, and E. coli*, respectively. Then, one colony of each strain was transferred into 10 mL of MRS broth incubated at 42 °C for 24h for *L. casei*, Malt Extract broth for *S. cerevisiae* and NB for *E. coli* strain. The assay was performed by adding 1% (vol/vol) of an overnight culture of each probiotic strain to separate tubes containing MRS broth for L. casei, Malt Extract broth for *S. cerevisiae*, with 1% (wt./vol) glucose or 1% (wt./vol) prebiotic inulin or OPMF extract at various concentrations (2, 4, 6, 8, 10% wt./vol). The cultures were incubated at 37 °C under anaerobic conditions (85% N, 10% CO, and 5% H) in an anaerobic jar for *L. casei*, and at 30 °C for *S. cerevisiae* under aerobic condition. Following the 0 and 24h of incubation, samples were counted on MRS agar using spread plate technique. For *S. cerevisiae*, 1% (vol/vol) of the overnight culture was added to separate tubes containing nutrient broth with 1% (wt./vol) glucose or 1% (wt./vol) prebiotic inulin or medium containing OPMF extract at various concentrations. The cultures were incubated at 32°C, and enumerated on malt extract agar after 1 and 24 h of incubation. For *E. coli*, 1% (vol/vol) of the overnight culture was inoculated to separate tubes containing Nutrient broth with 1% (wt./vol) glucose or inulin or OPMF extract at various concentrations. The cultures were incubated at 35 °C and counted on EMB agar after 0 and 24 h of incubation. Three replications were performed for each strain.

Prebiotic activity score (PAS)

PAS indicated the ability of a substrate to support the growth of probiotic *L. casei* or *S. cerevisiae* compared with that of enteropathogenic bacteria, *E. coli* in this study [Huebner et al., 2007]. Substrate has positive score indicated that it is metabolized as well as glucose by probiotic *L. casei* or *S. cerevisiae* but not by *E. coli*. Following equation was to calculate the PAS. The Equation 1 was used to calculate the prebiotic activity score (PAS) of a substrate:

RESULTS AND DISCUSSION

Extraction of OPMF

In this study, extraction of OPMF was for the procurement of cellulose. The process was performed through delignification, bleaching, and acid hydrolysis steps. Table 1 showed that the extraction of OPMF trough the delignification (10% NaOH) and acid hydrolysis (2% H_2SO_4) produced 31% material, which contained of 0.067% of reducing sugar and 34.14% of cellulose, and had an antioxidant activity of 38.37% measured with DPPH method. Similar finding in the research in cellulose isolation from OPMF [Megashah et al., 2018] found that multi step pretreatment using alkali and chlorine-free bleaching produced cellulose of 39.9 and 87.5% respectively. In addition, Al-Muraisy et al. (2017) reported that alkali pretreatment using 20% w/v NaOH at 70 °C and acid hydrolysis 20% v/v at 9 °C of Oil Palm Mesocarp Fiber (OPMF) produced 0.942 g/L of glucose. Whereas, research investigated by Chin et al. [2013] revealed that acid hydrolysis of oil palm empty fruit bunch (EFB) cellulose fibre at concentration of 4.63 N, at 133.7 °C for 2.05 h produced total reducing sugar of 39.81%.

Alkaline pretreatment (delignification) using NaOH aims to attack and break down the lignin structure within the oil palm mesocarp fibre, and transform an amorphous structure into a crystalline form, causing the cellulose structure to swell. This process enhances the crystalline structure of the oil palm fibre. The bleaching treatment using H_2O_2 on the cellulose fibres was to remove chromophore content from the remaining lignin after the delignification process [Andari et al., 2022]. Acid hydrolysis employed H_2SO_4 aims to cleave the polysaccharide chains in the oil palm mesocarp fibre and produce cellulose. The use of low concentration H_2SO_4 may prevent the possible formation of glucose through complete hydrolysis. Cellulose is a complex carbohydrate compound composed of many glucose chains which produces glucose monomers and cellobiose when hydrolysed. Cellulose is insoluble fibre that produces glucose monomers and some cellobiose when it was hydrolysed.

Growth (24h) of *S. cerevisiae*, *L. casei*, and *E. coli* on the media containing OPMF-extract at various concentrations

The ability of a substrate to stimulate the growth of probiotics, in this case *S. cerevisiae* and inhibit the growth of enteropathogenic intestines, in this case *E. coli*, is an indication that the substrate has the potential to be a prebiotic. Table 2 presented that the treatment significantly affected the growth of *S. cerevisiae, L. casei and E. coli* ($p < 0.5$). Further analysis indicated that the growth of *S. cerevisiae* on all of the media supplemented with OPMF-extract was not significantly different from on inulin, although P6 was better than the others (Table 2). In probiotic *L. casei*, P4, P5, P6, P7 were different from P3, inulin and glucose in supporting the growth of *L. casei*.

$$\mathrm{PAS} = \left(\frac{(probiotic \log\frac{cfu}{mL} on\ prebiotic\ at\ 24\ h - probiotic \log\frac{cfu}{mL} on\ prebiotic\ at\ 0h)}{(probiotic \log\frac{cfu}{mL} on\ glucose\ at\ 24\ h - probiotic \log\frac{cfu}{mL} on\ glucose\ at\ 0h)} - \frac{(E.coli \log\frac{cfu}{mL} on\ prebiotic\ at\ 24\ h - E.coli \log\frac{cfu}{mL} on\ prebiotic\ at\ 0h)}{(E.coli \log\frac{cfu}{mL} on\ glucose\ at\ 24\ h - E.coli \log\frac{cfu}{mL} on\ glucose\ at\ 0h)} \right) \quad (1)$$

Table 1. Chemical characteristic of cellulose extract from OPMF

Sample	OPMF flour	OPMF extract	Reducing sugar	Cellulose
OPMF (500 g)	100 g	31%	0.067%	34.14%

Note: data were the averages of triplicate.

Table 2. Effect of supplemented OPMF-extract on the growth (24 h) of *S. cerevisiae*, *L. casei* dan *E. coli*

Treatment	Total *S. cerevisiae* (Log CFU/mL)	Total *L. casei* (Log CFU/mL)	Total *E. coli* (Log CFU/mL)
P0 (growth medium)	7.32 ± 0.04^{a}	8.31 ± 0.3^{d}	5.37 ± 0.08^{b}
P1 (growth medium + 1% glucose)	8.51 ± 0.35^{c}	9.03 ± 0.08^{c}	6.1 ± 0.91^{a}
P2 (growth medium + 2% inulin)	8.44 ± 0.87^{bc}	9.14 ± 0.15^{bc}	4.258 ± 0.11^{d}
P3 (growth medium + 2% OPMF-extract)	7.75 ± 0.94^{abc}	9.13 ± 0.05^{c}	5.01 ± 0.29^{b}
P4 (growth medium + 4% OPMF-extract)	7.53 ± 0.49^{ab}	9.31 ± 0.48^{ab}	5.04 ± 0.31^{b}
P5 (growth medium + 6% OPMF-extract)	7.71 ± 0.81^{abc}	9.37 ± 0.09^{a}	4.96 ± 0.30^{bc}
P6 (growth medium + 8% OPMF-extract)	8.53 ± 0.51^{c}	9.39 ± 0.06^{a}	4.68 ± 0.70^{c}
P7 (growth medium + 10% OPMF-extract)	7.63 ± 0.96^{abc}	9.45 ± 0.02^{a}	4.67 ± 0.53^{cd}

Note: Data were the average of 3 replicates ± standard deviation. The values with different letters in the same column are significantly different ($P < 0.05$).

The concentrations of supplemented OPMF-extract to media did not affect in the growth of *L. casei*; even though, it was higher significantly in the media P4, P5, and P6 than media containing inulin. The concentration of supplemented OPMF-extract metabolized by probiotics were not significantly different, but the greater the prebiotic concentration increased the growth of probiotics. On the other hand, either prebiotic inulin or OPMF extract was not well supporting *E. coli*, when compared to its growth in glucose. A substrate has prebiotic activity if the substrate can be metabolized by probiotic test bacteria at or close to glucose metabolism by the bacteria, where glucose is the favorable growth media for *S. cerevisiae, L. casei and E. coli* [Chen et al., 2015].

Substrate containing of 8% OPMF-extract better supported the growth of *S. cerevisiae* than the other concentrations, since probiotic *S. cerevisiae* grew very well while the *E. coli* did not. The reason may relate to the production of β-glycosidase which is cellulose degradation enzyme in *S. cerevisiae*, which was not found in *E. coli*. β-glycosidase catalyzes the hydrolysis of glycosidic bonds such as 1,4- β and 1,6-α-linkages bond [Zhang et al., 2021, Liang et al., 2020]. The enzyme is produced both intracellular (cytosol, cell wall, and cell membrane) and extracellular (whole cell and yeast supernatant) with the latter enzyme has more activity and industrially valued. In addition, the low reducing sugar in OPMF (Table 1) may be unfavored for the growth of *E. coli*.

Similar work [Novianto et al., 2020] reported that as much as 60% peanut shell extract which likely contained cellulose added to growth medium, lignin and hemicellulose, could support the growth of *L. bulgaricus* (9.4×10^5 cfu/mL). Thus, OPMF may likely be considered to have the potential to be a prebiotic. Roberfroid [2007] stated that food ingredients containing oligosacchtzarides or polysaccharides (including dietary fiber) have potential as prebiotic.

Prebiotic activity

The prebiotic activity of a substrate is determined based on the difference's growth cells in Table 3. The result showed that substrate containing of OPMF-extract, inulin and glucose supported the of probiotic *L. casei* and *S. cerevisiae*

Table 3. Microbial loads between time 0 and 24 h, reported as log cfu/mL, for *E. coli, L. casei,* and *S. cerevisiae.*

Substrate	*E. coli* (log cfu/mL)			*L. casei* (log cfu/mL)			*S.cerevisiae* (log cfu/mL)		
	0 h	24 h	Δ	0 h	24 h	Δ	0 h	24 h	Δ
Glucose	5.37	6.17	0.79	8.31	9.03	0.72	7.32	8.51	1.19
Inulin	5.37	4.26	-1.12	8.31	9.14	0.82	7.32	8.44	1.12
PKE-extract 2%	5.37	5.01	-0.36	8.31	9.13	0.81	7.32	7.75	0.44
PKE-extract 4%	5.37	5.04	-0.33	8.31	9.31	1.00	7.32	7.52	0.21
PKE-extract 6%	5.37	4.97	-0.41	8.31	9.37	1.05	7.32	7.71	0.39
PKE-extract 8%	5.37	4.68	-0.69	8.31	9.39	1.07	7.32	8.54	1.22
PKE-extract 10%	5.37	4.67	-0.70	8.31	9.45	1.14	7.32	7.63	0.31

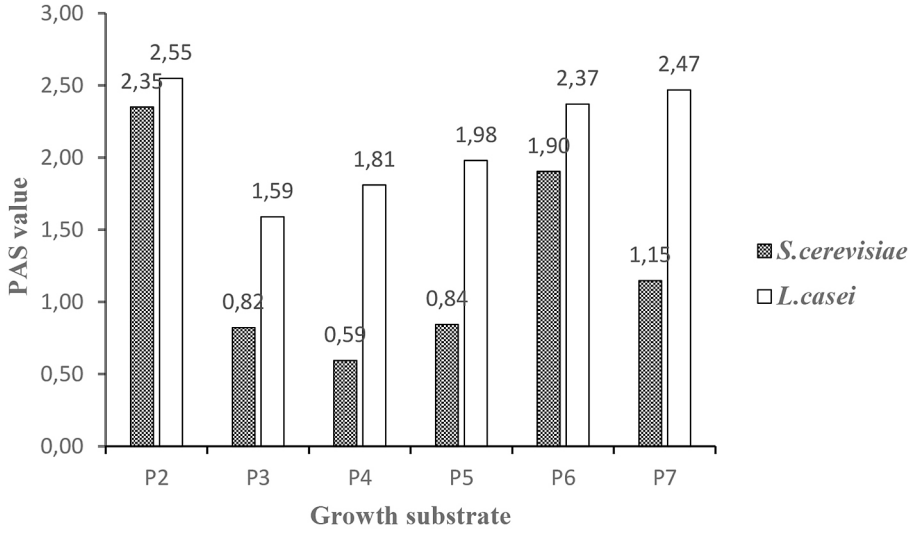

Figure 1. Prebiotic activity score of cellulose extracted from OPMF at various concentrations (P2 = inulin, P3, P4, P6, P8, and P10 was OPME-extract at 2, 4, 6, 8, and 10%, respectively)

(Fig. 1). The highest PAS value of *S. cerevisiae* and *L. casei* was 1.9 and 2.47, respectively when they grew on P6 (medium containing 8% of cellulose extract from OPMF) and P7 (10% of cellulose extract from OPMF) for *S. cerevisiae* and *L. casei*, respectively (Fig. 1). A low or negative PAS value is obtained if the growth of the test bacteria on the prebiotic is lower than the growth on glucose and/or the growth of the test bacteria on the prebiotic is lower than the growth of *E. coli* on the prebiotic. OPMF extract at all concentration tested could support the growth of *L. casei* at higher level than inulin, except 2% OPMF extract. The finding was not agreed with Phirom-on et al. [2021] found a negative value of prebiotic activity shown by *E. coli* which grew better in cellulose of banana peel extraction than *L. casei* TISTR1463 and *L. plantarum* TISTR2075. On the other hand, prebiotic index of *L. acidophillus* grown on extract of shallot, anion, and garlic was higher compare to that of on inulin [Moongngarm et al., 2011]. The reason for that could be due to different prebiotic sources and shelf capability of probiotic in metabolizing prebiotic.

The similar finding was reported by Marvie et al. [2021] that cellobiose, extraction of cassava tuber husk cellulose using cellulase, was found to have prebiotic potential with its ability to support the growth of *Lactobacillus plantarum* with a prebiotic activity value of 0.74. Meanwhile, Chen et al. [2015] stated that palm kernel expeller extract (PKE) oligosaccharides have the potential as a prebiotic for the growth of *Lactobacillus* and *Bifidobacteria* and inhibit *E. coli*. Type of prebiotic substrate for microbial growth and the ability of different strain probiotic in metabolizing specific prebiotic may cause low or negative PAS values. The results showed OPMF had positive value as prebiotic, although it was lower than inulin.

CONCLUSION

The supplementation of OPMF extract significantly supported the growth of both of probiotics used in this study ($p < 0.05$) similar to the prebiotic inulin. Different concentrations of OPMF extract did not influence the growth of *Saccharomyces cerevisiae* but its growth on 8% OPMF extract was higher than on glucose and inulin with the PAS value of 1.90. In addition, the growth of *L. casei* on OPMF extract at the minimal concentration of 6% and on inulin were higher significantly than on glucose, with the PAS in the range of 1.98–2.47. In contrast, the concentrations of OPMF extract had no significant effect on *Escherichia coli*, which showed minimal to no growth on the fiber substrate. OPMF as a source of prebiotics was very likely to be an important finding and was expected to contribute to the management of palm oil processing waste for food or feed.

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