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EFFECT OF *Pleurotus ostreatus* AQUEOUS EXTRACT ON PHYSICOCHEMICAL PROPERTIES, PROTEIN PROFILE AND TOTAL LACTIC ACID BACTERIA OF YOGURT FORTIFIED WITH *Lactobacillus acidophilus*

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ABSTRACT

Syneresis and low viscosity are two major quality defects found in yogurt. The addition of food stabilizer is necessary to overcome the issue. Oyster mushroom (*Pleurotus ostreatus*) could be utilized as a source of natural food stabilizer because it contains β-glucan. The objective of this study was to evaluate the effect of *Pleurotus ostreatus* aqueous extract (POAE) on physicochemical properties, protein profile and total lactic acid bacteria of yogurt fortified with *Lactobacillus acidophilus*. Yogurt was processed with the addition of POAE at 1%, 2%, and 3% (v/v) before fermentation and compared with control. Yogurt added with 3% POAE had the highest viscosity ($P<0.05$), protein content ($P<0.05$), and total acid content ($P<0.05$), but had the lowest syneresis ($P<0.05$) among others. The addition of POAE up to 3% (v/v), however, did not affect reducing sugar content and total lactic acid bacteria in yogurt. A 12.49 kDa protein was present in yogurt added with 3% POAE. Therefore, POAE could be used as natural stabilizer for yogurt fortified with *Lactobacillus acidophilus* with recommended addition level of 3% (v/v).

Keywords: Probiotic yogurt, oyster mushroom, stabilizer, syneresis, viscosity

INTRODUCTION

Yogurt is one of dairy products that offers health benefits. Yogurt is fermented by two well-known lactic acid bacteria (LAB), *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. The fortification of probiotics such as *Bifidobacterium animalis* and *Lactobacillus acidophilus* to yogurt has been reported to make yogurt being categorized as a functional food **(Lisko *et al.*, 2017)**. Probiotics are utilized for balancing gut microbiota and eventually preserves gut health **(von Martels *et al.*, 2017)**. **Marchesi *et al.* (2016)** mentioned that manipulating the composition of gut microbiota could potentially reduce the risk of metabolic syndrome, obesity-related disease, liver disease, inflammatory bowel disease and colorectal cancer through the supplementation of probiotics in diet.

Low viscosity and syneresis are two most common problems affecting the quality and acceptance of yogurt. These quality defects are the results of low final pH of yogurt. Besides uncontrolled fermentation conditions and storage time, the fortification of probiotics to yogurt could affect the acidity level and the pH of the final products. More organic acids are potentially produced by probiotics strain LAB such as *Lactobacillus acidophilus* **(Sarkar and Misra, 2010)**. The low pH condition causes the casein in yogurt losing its water-binding ability, promotes syneresis, degrades the firmness and lowers the acceptance **(Sawitri *et al.*, 2008)**. In order to avoid quality defects in yogurt caused by low pH condition, natural food stabilizer should be added **(Basiri *et al.*, 2018)**.

Food stabilizer is commonly made from starch, gelatin and protein concentrate **(Clark *et al.*, 2014)**. Yogurt made with food stabilizer has acceptable viscosity and no syneresis is found during display **(Skryplonek *et al.*, 2019)**. Oyster mushroom (*Pleurotus ostreatus*) is rich in β-glucan that can be used as natural food stabilizer **(Tjokrokusumo, 2010)**. β-glucan is one of dietary fibers that offers health benefits such as immunomodulator, reduce plasma cholesterol, prevent hypertension, diabetes, obesity and colorectal cancer **(Lambeau and McRorie, 2017)**. **Lazaridou *et al.*, (2014)** reported that β-glucan extracted from wheat improves viscosity and acceptance, reduces syneresis and calories without altering pH. The utilization of *Pleurotus ostreatus* has been studied in low-fat yogurt and yogurt drink **(Vital *et al.*, 2015; Anissa and Radiati, 2018)**. However, there is no information regarding the proper addition level of *Pleurotus ostreatus* in the form of aqueous extract to improve the quality of probiotics yogurt. Therefore, the objective of this study was to evaluate the effect of *Pleurotus ostreatus* aqueous extract (POAE) on physicochemical properties, protein profile and total lactic acid bacteria of yogurt fortified with *Lactobacillus acidophilus*.

MATERIALS AND METHODS

Pleurotus ostreatus aqueous extract preparation

The mushroom was soaked in water, rinsed and drained. Distilled water was added to mushroom (1:1, weight/weight) and homogenized for 10 min using food blender with maximum speed. The mushroom aqueous extract was pasteurized at 75°C for 30 min, cooled down to 45°C and filtered with cheesecloth. *Pleurotus ostreatus* aqueous extract (POAE) was immediately used for yogurt manufacture.

Yogurt preparation

Yogurt was made according to a method described by **Tamime and Robinson (2014)**. Briefly, whole milk obtained from Friesian Holstein cow was added with different levels of POAE (%, volume/volume); 1%, 2% and 3%, pasteurized at 85°C for 15 min, cooled down to 45°C. The milk was inoculated with 3% (weight/volume of milk) starter culture obtained from laboratory stock cultures, consisting of *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and *Lactobacillus acidophilus* with a ratio of 1:1:1. Liquid starter culture was diluted in 30 mL of pasteurized milk, added to the rest of pasteurized milk, and mixed for 10 min. The inoculated milk was incubated at 27°C for 18 h. Physicochemical and microbiological analyses were performed immediately after incubation.

Viscosity, total acid content, syneresis and pH measurement

Yogurt viscosity was measured using Brooke Field viscometer (AMTEK Brookfield, Middleboro, MA) with spindle No. 6 at 20 rpm. Yogurt was stirred for 5 min at room temperature (25±1°C). Viscosity (cP) was calculated as measurement unit shown in viscometer multiplied with 1000. Total acid content equivalent with lactic acid content was determined by titrating 20 mL of the samples (added with 2 drops of 1% phenolphthalein) with 0.1 N NaOH until the color changes into pink. Total acid content (%) was calculated according to AOAC method **(AOAC, 2005)**. Syneresis was determined using centrifugation method **(Robitaille *et al.*, 2009)**. Yogurt was weighed in a centrifuge tube and centrifuged at 1535 rpm for 20 min. The supernatant (whey released) was

weighed and syneresis was calculated as the weight percentage of whey released. The pH of the sample was determined in duplicate using a calibrated pH meter.

Protein and reducing sugar content measurement

Yogurt protein content was measured using Kjeldahl method **(AOAC, 2005)**. Crude protein was calculated as nitrogen content multiplied by 6.25. Reducing sugar content was determined using Luff-Schoorl reactant **(Marrubini *et al.*, 2017)**.

Lactic acid bacteria measurement

The number of lactic acid bacteria (LAB) was estimated by plating on MRS agar media. Plates were incubated at 30°C for 48 h, under anaerobic conditions. Total lactic acid bacteria were counted and expressed as log CFU/mL.

Protein profiling

Protein profile of the yogurt was identified based on their molecular weight using gel electrophoresis method **(Pisanu *et al.*, 2011)**. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed using a 15% polyacrylamide gel containing 30% acrylamide solution, 1.0 M Tris-HCl (pH 8.8), 10% SDS, 10% ammonium persulfate, and 10% N,N,N',N'-tetramethyl-ethylenediamine. The digesta sample was mixed with the same volume of sample buffer composed of 125 mM Tris-HCl (pH 6.8), 2% glycerol, 2% SDS, 2% mercaptoethanol, and 0.02% bromophenol blue, and heated at 95°C on a heating block for 90 sec. The 10 µL (74.7 µg protein) of a sample and the 5 µL of protein molecular markers (9-200 kDa) were loaded. Electrophoretic separation was performed with the pageRun system (AE-6531 mPAGE, ATTO Co., Tokyo, Japan) by applying 20 mA for 120 min. The running buffer was composed of 25 mM Tris, 0.1% SDS, and 192 mM glycine. Proteins in the gels were stained with Coomassie Brilliant Blue and then destained in a 10% acetic acid solution. Serum albumin (66.2 kDa), ovalbumin (45.0 kDa), carbonic anhydrase (31.0 kDa) dan lysozyme (14.0 kDa) were used as marker. The stained gel was scanned using a GS-710 (Bio-Rad Laboratories Inc, Hercules, CA, USA) densitometer at an optical resolution of 63.5 µm pixel-1.

Statistical analysis

This study employed a completely randomized design. The effect of POAE addition level (1%, 2%, and 3%, v/v) on physicochemical and total lactic acid bacteria of yogurt was observed using one-way analysis of variance. When significant, mean differences ($p<0.05$) were determined using least significant different test **(Subali, 2010)**.

RESULTS AND DISCUSSION

Physical properties

Analysis variance revealed that the viscosity, total acid content and syneresis of yogurt added with POAE at different level were significantly different ($P<0.05$). The mean differences among treatments and control are shown in Table 1. However, the pH of control and treatments were not significantly different.

Table 1 Viscosity, total acid content and syneresis of yogurt added with different levels of *Pleurotus ostreatus* aqueous extract

Variable	The addition level of *Pleurotus ostreatus* aqueous extract			
	0%	1%	2%	3%
Viscosity (cP)	613.25 ± 45.56^{a}	685.50 ± 27.14^{b}	756.50 ± 32.18^{c}	864.75 ± 20.90^{d}
Total acid content (%)	0.68 ± 0.03^{a}	0.72 ± 0.04^{a}	0.69 ± 0.02^{a}	0.78 ± 0.03^{b}
Syneresis (%)	56.77 ± 0.96^{c}	54.44 ± 1.28^{bc}	53.55 ± 1.33^{ab}	52.33 ± 2.53^{a}
pH	4.09 ± 0.03	4.11 ± 0.11	4.08 ± 0.10	4.01 ± 0.07

Legend: $^{a-d}$ Means with different superscripts were significantly different ($P<0.05$)

Viscosity is one of physical properties of most of liquid or semi-solid food, including yogurt that is responsible for consumer acceptance. The addition of POAE gave an effect on viscosity, in which the higher the addition level was, the higher the viscosity of yogurt was observed. The viscosity values of yogurt found in treatment groups were in the normal range set by national standard **(BSN, 2009)**. The national standard for the acceptable viscosity of yogurt ranges from 670 to 890 cP. The viscosity of the control, however, could not meet national standard. The viscosity of high-quality yogurt is between semi-solid and liquid. This is affected by the coagulation of water-binding protein in yogurt. **Wang *et al.* (2018)** mentioned that milk protein coagulation is caused by enzymatic reactions. The enzymes are derived from the metabolism of microorganism found in milk. In case of yogurt, *Streptococcus thermophilus* and *Lactobacillus bulgaricus* are responsible for protein coagulation. These LAB utilize lactose as carbon and energy sources during fermentation, producing metabolites such as lactic acid. Lactic acid decreases the pH of milk until reaching isoelectric point, the condition where protein coagulates. The acid condition triggers the separation of β-, α-, κ-, αs1-, αs2-casein and eventually causes the coagulation of casein **(Burton, 2014)**. **Widyastuti *et al.* (2011)** reported that the viscosity of POAE ranged from 11 to 19.70 cP. Therefore, POAE could improve the viscosity of yogurt **(Tamime and Robinson, 2014)**.

Yogurt has sour taste as it contains lactic acid that is produced by LAB during fermentation. According to national standard, the acceptable range of total acid content of yogurt is from 0.5 to 2.0% **(BSN, 2009)**. The total acid content (0.68%-0.78%) of yogurt found in this study met the national standard. Yogurt added with 3% POAE had the highest total acid content ($P<0.05$). POAE could be utilized by LAB as additional carbon source as POAE contains β-glucan, a water-soluble dietary fiber and a prebiotic **(Sari *et al.*, 2017; Lam *et al.*, 2019)**. This could increase the production of acid by LAB, thus the total acid content of yogurt added with 3% POAE was higher than the others.

Syneresis is one of quality defects found in yogurt. The pH, total acid content and water-holding capacity influence the occurrence of syneresis in yogurt **(Dönmez *et al.*, 2017)**. The addition of POAE to the yogurt reduced syneresis susceptibility. Yogurt added with 3% POAE had the lowest syneresis ($P<0.05$). In this study, POAE was found to possess the functional characteristics of food stabilizer as POAE was responsible to increase viscosity and reduce syneresis. PO has been well known containing β-glucan **(Sari *et al.*, 2017)**. This water-soluble dietary fiber has been observed having water-binding properties and improving the rheological characteristics of full fat yogurt **(Kaur and Riar, 2020)**.

POAE plays a role as energy source for LAB to grow and the metabolites such as lactic acid decrease the pH of yogurt. **Syainah *et al.* (2014)** mentioned that the higher the total lactic acid bacteria present in yogurt, the lower the pH and the higher the acidity level are observed. **Akiyama *et al.* (2019)** reported that pH value is negatively associated with total acid content of yogurt. In this study, there were no significant differences found on pH value of the yogurt. The observed pH value ranged from 4.01 to 4.11 (lower than casein isoelectric point). **Francis *et al.* (2019)** mentioned that the isoelectric point of the casein ranges from pH 4.3 to pH 4.7. The fortification of probiotics to yogurt in this study could lower the pH value of the final products. More organic acids are potentially produced by *Lactobacillus acidophilus* **(Sarkar and Misra, 2010)**.

Protein and reducing sugar content

Table 2 shows that the protein content of yogurt added with POAE at different level was significantly different ($P<0.05$). The protein content of yogurt depends on the quality of the milk or protein content of skim milk. According to national standard, acceptable yogurt should contain at least 2.7% of protein **(BSN, 2009)**. In this study, the addition of POAE positively impacted the protein content of the yogurt as the protein content of POAE contributed to the additional amount of protein in yogurt. POAE in this study contained 31.02 ± 0.92 of protein (data are not shown).

Table 2 Protein and reducing sugar content of yogurt added with different levels of *Pleurotus ostreatus* aqueous extract

Variable	The addition level of *Pleurotus ostreatus* aqueous extract			
	0%	1%	2%	3%
Protein (%)	2.63 ± 0.01^{a}	2.73 ± 0.01^{b}	2.79 ± 0.00^{c}	2.92 ± 0.01^{d}
Reducing sugar (%)	4.91 ± 0.29	5.55 ± 1.08	5.56 ± 1.08	6.33 ± 0.76

Legend: $^{a-d}$ Means with different superscripts were significantly different ($P<0.05$)

No significant differences were observed on reducing sugar content in yogurt added with POAE. High reducing sugar content in yogurt added with POAE was caused by the carbohydrate present in POAE. **Umi *et al.* (2013)** mentioned that 25%-30% of monosaccharides in milk are hydrolyzed by LAB during fermentation. Lactose in milk is not easily digested in human intestine, however, it can be digested by gut LAB **(Forsgård, 2019)**. LAB produces β-D-

galaktosidase to hydrolize lactose and anaerobic glycolysis results in lactic acid, acetic acid and some other volatile organic acids **(Hidayat *et al*., 2013)**. Thus, yogurt is more likely suitable for those who have lactose-intolerant.

Total lactic acid bacteria

Table 3 shows that total LAB in yogurt added with POAE at different level was not different significantly. The viability of LAB in yogurt is shown by the number of these bacteria, pH, lactose content and total acid content **(Syainah *et al*., 2014)**. The acceptable range of total LAB in yogurt is 7 log CFU/mL **(BSN, 2009)**. The addition of POAE contributed to the viability of LAB as POAE could be used as carbon and energy sources. β-glucan derived from POAE is a prebiotic that supports the viability of LAB in yogurt. Therefore, total LAB in treatment groups was within the standard range.

Table 3 Total lactic acid bacteria in yogurt added with different levels of *Pleurotus ostreatus* aqueous extract

The addition level of *Pleurotus ostreatus* aqueous extract	Total lactic acid bacteria (log CFU/mL)
0%	6.85 ± 0.10
1%	6.86 ± 0.21
2%	7.02 ± 0.07
3%	7.06 ± 0.07

Protein profile

SDS-PAGE revealed protein bands derived from yogurt added with POAE (Figure 1). There were six fractions with different molecular weights found in all groups except for yogurt added with 3% POAE. The highest addition level (3%) resulted in the presence of fraction with molecular weight of 12.49 kDa. The other six fractions' weight ranged from 10.41 kDa to 146.49 kDa (Table 4). **Karitas and Fatchiyah (2013)** mentioned that the molecular weight of α-casein, β-casein and κ-casein ranged from 30 to 60 kDa. **Susanti and Hidayat (2016)** mentioned that bacterial fermentation is responsible for the degradation of macro molecules in food into novel micro molecules. The presence of observed fractions figures out the degradation of milk major protein during fermentation. Furthermore, the addition of POAE at 3% (v/v) to yogurt resulted in the presence of unique fraction with molecular weight of 12.49 kDa.

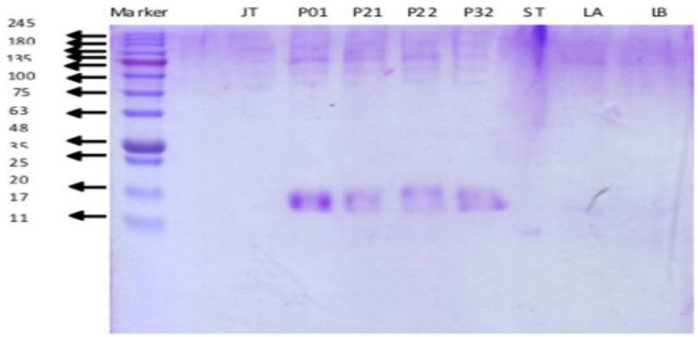

Figure 1 SDS PAGE gel showing protein derived from yogurt added with different levels of *Pleurotus ostreatus* aqueous extract (POAE). JT: *Pleurotus ostreatus* aqueous extract, P01: Control, P12: Yogurt + 1% POAE, P22: yogurt + 2% POAE, P32: Yogurt + 3% POAE, *Streptococcus thermophilus* (ST), *Lactobacillus acidophilus* (LA), *Lactobacillus bulgaricus* (LB).

Table 4 Molecular weight of protein derived from yogurt added with different levels of *Pleurotus ostreatus* aqueous extract

No.	Molecular weight (kDa)			
	Pleurotus ostreatus aqueous extract addition level			
	0%	1%	2%	3%
1	146.49	146.49	146.49	146.49
2	122.07	122.07	122.07	122.07
3	101.72	101.72	101.72	101.72
4	84.76	84.76	84.76	84.76
5	64.48	64.48	64.48	64.48
6	10.41	10.41	10.41	12.49
7	-	-	-	10.41

CONCLUSION

Pleurotus ostreatus aqueous extract can be utilized as stabilizer to improve viscosity and reduce syneresis of yogurt fortified with *Lactobacillus acidophilus*. The addition level of POAE at 3% (volume/volume) is recommended.

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