

THE EFFECT OF DIFFERENT STARTER CULTURES ON THE FERMENTATION OF YOGURT ADDED WITH AQUEOUS EXTRACT OF WHITE OYSTER MUSHROOM (*Pleurotus ostreatus*)

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ABSTRACT

The objective of this study was to evaluate the effect of different starter cultures on the fermentation of yogurt added with aqueous extract of white oyster mushroom (*Pleurotus ostreatus*) whether each starter culture respond differently. This study used a completely randomized design (CRD) with 3 treatments and 4 replications. Yogurt was made with one of the starter cultures as treatment: *Streptococcus thermophilus* (ST), *Lactobacillus acidophilus* (LA), or *Lactobacillus bulgaricus* (LB). Data were analyzed using analysis of variance followed by the least significant difference test. The variables tested were pH, total acid content, viscosity and total lactic acid bacteria. The results showed that yogurt made with ST had lower pH and total acid than the others. The use of LB resulted in higher total acid content. The addition of different starter cultures in yogurt did not influence viscosity. Among starter cultures, the use of LA resulted in higher total lactic acid bacteria (LAB) than the others. However, the number of LAB from different treatments were not much different (within the range of 7 log CFU/mL). It is suggested that the three starter cultures are adaptive and capable to fermenting yogurt added with aqueous extract of white oyster mushroom (*Pleurotus ostreatus*).

Key words: Fermentation; mono starter; oyster mushroom; starter activity

INTRODUCTION

Yogurt is one of fermented dairy products that offers health benefits for human. It is produced through the fermentation process carried out by lactic acid bacteria (LAB), *Streptococcus sulvarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. The fortification of *Lactobacillus acidophilus*, a probiotic LAB, to yogurt has been also applied to produce probiotic yogurt (Lisko et al., 2017). The quality of yogurt depends on the quality of milk, fermentation time, incubation temperature, and starter cultures (Hapsah and Astriana, 2012).

During fermentation, the cell of LAB converts lactose in milk into organic acids, e.g. lactic acid, and energy, thus reducing pH of the yogurt (Afriani, 2010). Yogurt can also be fortified with other functional ingredients that can improve the quality and nutrition value of yogurt. The acidity level of yogurt and storage conditions, in some cases, can reduce the viscosity of yogurt, resulting the appearance of syneresis or whey released. In order to maintain the acceptable viscosity of yogurt during storage, food stabilizer is added into the formulation. Food stabilizer for dairy products is made from protein concentrate, gelatin, starch and other polysaccharides (Clark et al., 2014). Yogurt, which is made with food stabilizer, has acceptable viscosity without syneresis during display (Skryplonek et al., 2019). Food stabilizer, which is made from polysaccharide, can play a role as prebiotic too. White oyster mushroom (*Pleurotus ostreatus*) can be utilized to produce natural food stabilizer as it contains β -glucan (Tjokrokusumo, 2010).

β -glucan derived from mushroom is one of dietary fibers that offers health benefits for human as it plays a role as prebiotic in human gut (Widyastuti et al., 2011). Pranamuda et al. (2012) mentioned that 164 g of β -glucan can be extracted from 28 kg of *Pleurotus ostreatus*.

Many studies have revealed the functionality of β -glucan, e.g. as immunomodulator, reduce plasma cholesterol, prevent colorectal cancer, hypertension, obesity and diabetes (Lambeau and McRorie, 2017). Lazaridou et al. (2014) reported that β -glucan extracted from wheat improves acceptability and viscosity, prevents syneresis and reduces calories without altering the pH of yogurt. The utilization of *Pleurotus ostreatus* has been studied previously in low-fat yogurt and yogurt drink (Vital et al., 2015; Anissa and Radiati, 2018).

However, there is no information regarding the ability of LAB to fermenting yogurt containing aqueous extract of *Pleurotus ostreatus*. Therefore, the objective of this study was to evaluate the effect of different starter cultures on the fermentation of yogurt added with aqueous extract of white oyster mushroom (*Pleurotus ostreatus*) whether each starter culture respond differently.

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 slurry was pasteurized at 75°C for 30 min, cooled

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down to 45°C and filtered with filter cloth. *Pleurotus ostreatus* aqueous extract was immediately used for yogurt manufacture.

Yogurt preparation

Yogurt was made according to a method described by Tamime and Robinson (2014). Briefly, milk obtained from Friesian Holstein cow was added with different levels of *Pleurotus ostreatus* aqueous extract at 3% (volume/volume), pasteurized at 85°C for 15 min, cooled down to 45°C. Each starter culture: *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and *Lactobacillus acidophilus*, was diluted in 30 mL of pasteurized milk, added to the rest of pasteurized milk separately, 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 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. 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). The pH of the sample was determined in duplicate using a calibrated pH meter.

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.

Statistical analysis

This study employed a completely randomized design. The effect of different starter cultures on the fermentation of yogurt added with *Pleurotus ostreatus* aqueous extract 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

pH value

The pH value of the yogurt added with *Pleurotus ostreatus* aqueous extract and fermented by different starter cultures was significantly different ($P < 0.01$). Yogurt fermented by *Streptococcus thermophilus* had the lowest pH among others. While, the pH of yogurt fermented by *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* showed no differences. Dahlan and Sani (2017) reported that the fermentation rate of *Streptococcus thermophilus* is faster than other LAB used as yogurt starter culture. The pH of *Pleurotus ostreatus* aqueous extract and milk used for yogurt production in this study was 4.8 and 6.5, respectively. Burton *et al.* (2014) mentioned that pH value reflects the amount of ion H^+ from organic acids in fermented milk as a result of bacterial growth.

In preliminary study, the addition of a combined starter cultures consisting of *Lactobacillus bulgaricus*, *Streptococcus thermophilus* and *Lactobacillus acidophilus* to yogurt added with *Pleurotus ostreatus* aqueous extract resulted in pH ranged from 4.0 to 4.1, which is lower than the pH value found in present study. *Streptococcus thermophilus* plays a role to convert lactose into lactic acid.

However, it requires valine to promote the growth of *Streptococcus thermophilus*. Valine is an amino acid produced by *Lactobacillus bulgaricus* during fermentation as one of its metabolites (Burton *et al.*, 2014). Therefore, the addition

of mono starter in yogurt production does not optimize the fermentation of milk.

Total acid content

The total acid content of the yogurt added with *Pleurotus ostreatus* aqueous extract and fermented by different starter cultures was significantly different ($P<0.01$). Yogurt fermented by *Lactobacillus bulgaricus* had the highest total acid content, followed by that fermented by *Lactobacillus acidophilus* and *Streptococcus thermophilus*. Microorganisms require energy derived from carbohydrate, protein and fat found in food. The production rate of lactic acid during

fermentation is influenced by the species of starter culture (Afriani, 2010). Fatmawati *et al.* (2013) mentioned that *Lactobacillus bulgaricus* converts lactose in milk into lactic acid.

The addition of carbohydrate source, e.g. *Pleurotus ostreatus* aqueous extract, in milk could possibly optimize the fermentation of yogurt by mono starter culture. However, the amount of acid produced was less than 0.4%, which is not enough for achieving the yogurt's typical tangy taste. The acceptable range of total acid content in yogurt is between 0.5% to 2.0% according national standard (BSN, 2009).

Table 1. The pH, total acid content, viscosity and total lactic acid bacteria of yogurt added with *Pleurotus ostreatus* aqueous extract and fermented by different starter cultures

Variable	Species		
	<i>Streptococcus thermophilus</i>	<i>Lactobacillus acidophilus</i>	<i>Lactobacillus bulgaricus</i>
pH	4.60±0.14 ^a	4.87±0.03 ^b	4.88±0.04 ^b
Total acid content (%)	0.21±0.01 ^a	0.33±0.02 ^b	0.37±0.01 ^c
Viscosity (cP)	14.5±1.91 ^a	20.5±2.08 ^a	10.0±1.63 ^a
Total lactic acid bacteria (log CFU/g)	7.37±0.04 ^a	7.52±0.02 ^c	7.38±0.18 ^b

Data are shown as mean±standard deviation. Different superscripts in the same column indicate a highly significant effect ($P<0.01$).

Viscosity

The viscosity of the yogurt added with *Pleurotus ostreatus* aqueous extract and fermented by different starter cultures was not significantly different. The viscosity of yogurt is affected by pH, protein content, starter culture strain, incubation time and milk total solid content (Purbasari *et al.*, 2014). This suggests that the addition of mono starter; *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and *Lactobacillus acidophilus* in yogurt added with *Pleurotus ostreatus* aqueous extract does not optimize the fermentation as the viscosity of yogurt in this study is close to the viscosity of water (1 cP at 25°C).

Total lactic acid bacteria

The total lactic acid bacteria in the yogurt added with *Pleurotus ostreatus* aqueous extract and fermented by different

starter cultures was significantly different ($P<0.01$). Yogurt fermented by *Lactobacillus acidophilus* had the highest total lactic acid bacteria, followed by that fermented by *Lactobacillus bulgaricus* and *Streptococcus thermophilus*.

Total lactic acid bacteria is an indicator of microbiological quality of yogurt. The growth of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in yogurt is associated with symbiosis mutualism. These bacteria did not grow as well as *Lactobacillus acidophilus* when being used as mono starter for yogurt production. *Lactobacillus acidophilus* could grow independently resulting a higher number of lactic acid bacteria than other treatments. Although the number was significantly different, the range was still around 7 log CFU/g. The number of lactic acid bacteria in this study is in line with the

national standard, i.e. 7 log CFU/g (BSN, 2009). This indicates that *Streptococcus thermophilus*, *Lactobacillus acidophilus*, and *Lactobacillus bulgaricus* are adaptive and capable to fermenting yogurt added with *Pleurotus ostreatus* aqueous extract.

CONCLUSION

Three different starter cultures used in this study; *Streptococcus thermophilus*, *Lactobacillus acidophilus*, and *Lactobacillus bulgaricus* are adaptive and capable to fermenting yogurt added with *Pleurotus ostreatus* aqueous extract. The fermentation of yogurt added with *Pleurotus ostreatus* aqueous extract using three different mono starter cultures resulted in different yogurt characteristics in terms of acidity, viscosity and total lactic acid bacteria.

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