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The changes of sesaminol triglucoside and antioxidant properties during fermentation of sesame milk by *Lactobacillus plantarum* Dad 13

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Abstract

Sesame milk fermentation using *L. plantarum* Dad 13 was tested to study its ability to hydrolyze sesaminol triglucoside to aglycone. Changes in growth, pH, titratable acidity (TA), sugar concentration, organic acid production, β-glucosidase activity, DPPH radical scavenging activity and total phenolic content were investigated during fermentation on sesame milk at 37°C for 18 h. Changes of sesaminol triglucoside was analyzed using HPLC (High Pressure Liquid Chromatography). The antioxidant properties were evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. The result showed that higher viable counts were obtained after 18 h (8.88 log $CFUmL^{-1}$) followed by a drop of pH, an increase of acidity during fermentation due to the production of organic acids, DPPH radical scavenging activity and total phenolic content. *Lactobacillus plantarum* Dad 13 was able to proliferate in sesame milk and produced a high β-glucosidase activity (71.07 ± 0.9 mU/mL sesame milk). Sesaminol triglucoside decreased from 11.72 mg/100 mL to 5.11 mg/100 mL of sesame milk. The DPPH radical scavenging activity increased 2.55 times after fermentation. The present study indicates that *L. plantarum* Dad 13 was able to transform sesaminol triglucoside to sesaminol aglycone and could be used in the development of sesame yoghurt-like beverages and improve the food functionality of sesame milk.

Introduction

Sesame seed *(Sesamum indicum)* is well-known as a source of high antioxidant activity (Shyu *et al.*, 2002; Shahidi *et al.*, 2006). Many scientific studies have been conducted to investigate the health-promoting effects of sesame (Hirose *et al.*, 1992; Akimoto *et al.*, 1993; Matsumura *et al.*, 1995; Chavali *et al.*, 1998; Hou *et al.*, 2003; Hou *et al.*, 2004).

Decorticated sesame seeds contain oil (48-55%), protein (20%), carbohydrate (14%), and ash (3%). Tocopherol and lignans are the bioactive compounds which are found in sesame seeds. Lignans has higher antioxidant activity than Tocopherol (Fukuda *et al.*, 1985). Sesame seed naturally contains lignans and lignan-glucosides as the functional components which mainly related to its antioxidant properties. The major lignans in sesame seed are hydrophobic sesamin and sesamolin (Kamal and Appelqvist, 1994). However, among the sesame lignans, sesaminol, was reported as the most effective antioxidant activity in in-vitro experimental systems (Kang *et al.*, 2000). Moreover, sesaminol has reported as an inhibitor to inflammatory hepatic ischemia-reperfusion injury in rats. Meanwhile, lignan-glucosides which are exist mainly in the defatted sesame meal, there are hydrophilic antioxidant. The primary lignan-glucosides in sesame are sesaminol-glucoside, pinoresinol-glucoside, and sesamolinol-glucoside. In a recent study, a dietary sesaminol-glucosides were found to inhibit the development of colonic pre-cancerous lesions *in vivo* (Sheng *et al.*, 2007).

Almost 32% lignans in sesame seeds present in a glycosylated form. Although the lignan glucosides have no direct role in antioxidative defense system, the intestinal β-glucosidase may hydrolyze the lignan-glucosides complex to free-form lignan and aglycone, which results in the improvement of the antioxidant properties of the lignan-glucosides itself.

Sesaminol triglucosides are the major lignan-glucosides in sesame seeds (Ryu *et al.*, 1998; Shyu and Wang, 2002; Moazzami *et al.*, 2006). Another sesaminol glucosides, in particular sesaminol mono- and di-glucoside, were reported as resistant to intestinal β-glucosidase regarding to the sugar moieties position which are very close to the sesaminol aromatic ring, made a steric hindrance to the active site of the enzyme. In contrast, sesaminol triglucoside was able to become a substrate of β-glucosidase, although the reaction mechanism

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remains unclear (Katsuzaki *et al*., 1994).

Much attentions have been addressed to vegetable based beverages due to its nutritious and health beneficial properties. The processing of sesame seed itself as vegetable milk has been reported by Quasem *et al.* (2009) with a positive sensory acceptance. However, the panelist reported the presence of a specific flavor sensation, which is disliked. This unfavored flavor has been successfully covered by the flavor resulted by lactic acid fermentation (Afaneh *et al.*, 2011). Lactic acid fermentation of vegetable milk may offer another beneficial properties. Fermentation of soymilk using lactic acid bacteria have been reported to enhance the antioxidant activities content due to the formation of isoflavone aglycones (Tsangalis *et al*., 2002; Pyo *et al*., 2005; Chun *et al.*, 2007, Djaafar *et al*., 2013). This phenomenon was associated with the production of β-glucosidase by lactic acid bacteria. Beta glucosidase activity of some strains of *L. plantarum* in fermentation of kerandang *(Canavalia virosa)* milk reduced main isoflavone glucoside which was followed by the increasing of aglycone content, indicated a hydrolysis reaction catalyzed by β-glucosidase-producing bacteria (Djaafar *et al.*, 2013).

The ability of several *L. plantarum* strains to increase aglycone isoflavone content in fermented soymilk has been reported (Otieno *et al.*, 2006; Wei *et al*., 2007; Chun *et al*., 2007). *Lactobacillus plantarum* subsp. *pentosus* T14 was able to transform isoflavone glucoside to aglycone in fermentation of kerandang milk (Djaafar *et al*., 2013). And also *L. plantarum* KFRI 00144 was able to transform isoflavone glucoside to aglycone in fermentation of soymilk. The present study was investigated a possible application of a functional starter cultures of β-glucosidase-producing lactic acid bacteria in sesame milk fermentation to obtain the bioactive lignan aglycone, sesaminol.

Much attention was addressed to *L. plantarum* Dad 13 which was isolated from dadih, a traditional fermentation product of buffalo milk from West Sumatra, Indonesia. Health benefits of *L. plantarum* Dad 13 was already reported, such as a lowering effect of total cholesterol, HDL, triglyceride, and LDL/HDL ratio in mice (Lestari *et al*., 2003). Therefore, in this study, fermentation of sesame seed by *L. plantarum* Dad 13 to increase the level of sesaminol aglycones and antioxidant properties were conducted. The objectives of this research was evaluation sesaminol aglycones level and antioxidant activity in sesame milk fermented by *L. plantarum* Dad 13, a β-glucosidase-producing bacteria.

Material and Methods

Sesame seeds

Decorticated sesame seeds of Winas variety were obtained from Brajan Village, Prambanan District, Klaten Regency, Indonesia. The saccharides and sesaminol triglucoside in sesame milk were analyzed by HPLC. Sugar standards including glucose, fructose, galactose, sucrose, stachyose and raffinose were all obtain from Sigma Chemical Compani (St. Louis, Missouri, USA). Naringenin was used as internal standar for determination of sesaminol triglucoside in sesame milk (Sigma-Aldrich, Singapore).

Preparation of sesame milk

Sesame milk was prepared according to Quassem *et al*. (2009) using 12% (w/v) initial concentration of sesame seed.

Bacterial strain and growth condition

Lactobacillus plantarum Dad 13 was obtained from FNCC (Food Nutrition Culture Collection) Centre for Food and Nutrition Studies, Gadjah Mada University, Yogyakarta. The culture stock was kept in 10% glycerol and 10% skim milk with the ratio 1:1 (v/v). One mililiter culture in sterile 1.5 mL polyethylene screw cap tube was added with 1 mL glycerol-skim milk and stored at -40°C. The strain was activated by adding 1 mL of stock solution with 9 mL of 0.85% NaCl in water, vortex and then rejuvenated in 10 mL of MRS (De mann Rogosa Sharpe) broth (Oxoid) at 37°C for 18 h.

Fermentation of sesame milk

Sesame milk in glass bottle were pasteurized at 75°C for 5 min in autoclave (EYELA MAC 5160), Tiyoda IX Manufacturing Ltd, Japan). The pasteurized milk was inoculated aseptically with an active single culture of *L. plantarum* Dad 13 1% (v/v) and incubated at 37°C for 18 h. Viable cell, pH, titratable acidity, and β-glucosidase were determined every 3 h during fermentation. Lactic acid, acetic acid, sesaminol triglucoside, and sugar concentration were determined every 6 h during fermentation. Sesame milk fermentation was stored at -20°C immediately and than freeze dried using a Dynavac FD 300 freeze drier (Rowville, Vic., Australia) for the analysis of lactic acid, acetic acid, sugars and sesaminol triglucoside.

Enumeration of bacterial population

Cell number was measured in triplicate using pour plate method (Donkor *et al.* 2005) with lactobacilli

MRS media (Oxoid). Fermented sample (1 mL) was serially diluted with 0.85% NaCl solution, and then 100 µL of diluted samples were taken into sterile plates. MRS medium containing 1.5% agar and 0.8% $CaCO_3$ was poured into the plate and mixed carefully. After incubation at 37°C for 48 h, single colony was counted.

pH measurements

Change in pH were monitored during fermentation of sesame milk at 0,3,6,9,12 and 18 h using pH meter (Thermo scientific, Orion 3 start) at 27°C after calibration with fresh standard buffer pH 4.0 and 7.0.

Titratable acidity

The titratable acidity was measured by titration of fermented sesame milk with 0,1 N NaOH using 1% phenolphtalein as indicator. Titratable acidity was calculated and expressed as percent of lactic acid.

Determination of β-glucosidase activity in fermented sesame milk

Beta-glucosidase activity was determined by measuring hydrolysis rate of ρ-nitrophenyl-β-D-glucopyranoside (ρNPG) (Sigma Chemical Co., St. Louis, Mo., USA). One mililiter of 5 mM ρNPG prepared in 100 mM sodium phosphate buffer (pH 7.0) was added to 10 mL of each aliquot and incubated at 37°C for 30 min. The reaction was stopped by the addition of 500 µL of 1 M cold sodium carbonate (4°C) (Scalabrini *et al*., 1998). The aliquots were then placed in a 1.5 mL effendorf centrifuge tubes followed by centrifugation (14,000 g for 30 min). The amount of ρ-nitrophenol released was measured by spectrophotometer UV-Vis (Shimadzu, UV-1656 PC) at 420 nm. One unit of the enzyme activity was defined as the amount of β-glucosidase that release 1.0 nmol of p-nitrophenol from the substrate ρ-NPG per mililiter per min under assay conditions. Para nitrophenol was used as standard in the enzyme assay.

Determination of organic acids

The main metabolic products are organic acids, particularly lactic acid and acetic acids. The concentration of these acids were measured according to the method of Donkor *et al*. (2005) using high-performance liquid chromatography (HPLC). Samples were analyzed by HPLC (Waters), Binary 1525 EF model pump, with a photodiode array detector (PDA) 2996 which also provided the UV Spectra of the peaks and an Phenomenex® Luna C 18 (250 x 4.60 mm, 5 µm), Alltech Co., Waukegan Road, Deerfield, IL). The mobile phase was 0.008 M H_2SO_4 with a flow rate set at 1 mL /min and the temperature of the column was set at 35°C. Organic acids were detected at 220 nm. For determination of organic acids, 2.5 g samples were mixed with 50 µL of 15.8 N HNO_3 and 1.0 mL of 0.001 M H_2SO_4 before subjecting a 1.5 mL aliquot of the mixture to the centrifugation at 14000 x g for 30 min at room temperature. The supernatant was filtered through a 0.45 µm membrane filter (Schleicher and Schvell, Germany) and 20 µL of the filtrate was injected into HPLC system.

Sugars analysis

The HPLC method described by Matsuyama *et al.* (1992) was used to determine the contents of sugar. One mililiter of samples mixed with 10.0 mL of deionized distilled water and centrifugated for 30 min at 17,000 rpm. Supernatant fractions were filtered through a 0.45 µm membrane (Gelman sciences, Pall Co., Ann Arbor, Michigan, USA). Samples were analyzed by HPLC (Waters), Binary 1525 EF model pump, with a photodiode array detector (PDA) 2996 which also provided the UV Spectra of the peaks and an Phenomenex® Luna C 18 (250 x 4.60 mm, 5 µm), Alltech Co., Waukegan Road, Deerfield, IL). When samples were analyzed for oligosaccharides, the mobile phase consisted of 65% acetonitrile (Mallinck-rodt Baker Inc., Phillipsburg, New Jersey USA) in deionized distilled water. The flow rate and column temperature where 2.0 mL min^{-1} and 40°C respectively. For disaccharide and monosaccharides determination, the mobile phase consisted of 85% acetonitrile and the flow rate was 0.8 $mLmin^{-1}$.

Sesaminol triglucoside analysis

The extraction of sesaminol triglucosides from freeze-dried fermented sesame milk was performed in triplicate, according to the methods of Moazzami *et al.* (2006). Freeze-dried sesame milk sample was weighed (0.5 g) in glass tube (35 mL) and extracted for 5 h with 8.25 mL of 85% ethanol containing 100 µg/mL of naringenin as internal standard. Then, the extraction was continued overnight after adjusting the ethanol concentration to 70% by adding 1.75 mL of distilled water to the extraction tubes. Thereafter, the tubes were centrifuged for 10 min at 2000 rpm and the supernatants were filtered (0.45 µm PTEF membrane, Pall acrodise, Ann Arbor, MI).

Samples were analyzed by HPLC (Waters), Binary 1525 EF model pump, with a photodiode array detector (PDA) 2996 which also provided the UV Spectra of the peaks and an Phenomenex® Luna C 18 (250 x 4.60 mm, 5 µm), Alltech Co., Waukegan Road, Deerfield, IL). The eluents were used (A) 0.01

M phosphat buffer (pH 2.8) containing 5% acetonitrile and (B) acetonitrile. The elution conditions were 0-5 min (15% B), 30 min (30% B), and 40-50 (70% B) and the flow rate was 1.0 mL/min.

The quantity of sesaminol triglucoside was calculated from the peak area at 290 nm against the internal standard (naringenin). To calculate the relative response factors (RRFs) with reference to the internal standard, solution including 10 different concentrations of sesaminol triglucoside and naringenin were analyzed by HPLC and peak areas that were used to draw the calibration curve for sesaminol and naringenin. The respond factor for sesaminol relative to naringenin was calculated as 0.0008 and was used to calculate the concentration of sesaminol triglucoside in dried sesame milk fermented, assuming equal response.

Preparation of crude extract of sesame milk and sesame milk fermentation

One mililiter of sesame milk or fermented sesame milk was transferred into flasks and 5 mL of methanol 70% were added into the solution. The flasks were then placed in shaker (120 rpm) for 1 h in room temperature, followed by maceration at 4°C for 24 h. The extracts were obtained by centrifugation at 4000 g 4°C for 10 min and filtration through a Whatman paper no 42. The volume was measured. The extraction was repeated twice and the supernatants obtained were combined together (Xu and Chang, 2007). The extraction was stored at -20°C until analysis for determination of DPPH radical scavenging activity and total phenolic compounds.

Determination of DPPH radical scavenging activity

Experiments were carried out according to Wang *et al.*, (2007). Briefly, 0.06 mM DPPH solution in methanol was prepared, and then 3 mL DPPH solution was mixed with 1 mL extract of sesame milk and sesame milk fermentation. Incubated for 1 h in the dark room. The absorbance was measured at 516 nm by a Shimadzu UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan). Decrease of the absorbance of the DPPH solution indicates the existance of DPPH radical scavenging activity. This activity is given as percent DPPH radical scavenging which is calculated with following equation.

$$\text{Scavenging activity \%} = \left[\frac{control\ absorbance - sample\ absorbance}{control\ absorbance}\right] X\ 100\%$$

The control contained 3 mL of 0.06 mM DPPH solution and 1 mLof methanol. Ascorbic acid was used as positive controls. Data were reported as means ± SD for three replications.

Analysis of total phenolic content

The total phenolic content in the crude extract of sesame milk and sesame milk fermentation was determined according to the Folin-Ciocalteu procedures (Singleton and Rossi, 1965). In brief, 2 mL extract was placed in a tube, so 1 mL Folin-Ciocalteu reagents was added, mixed, and allowed to stand for 1 min. Then 4 mL of 15% of sodium carbonate (Na_2CO_3) solution were added, mixed, and placed in a dark room for 2 h at room temperature. Absorbance of the resulting blue complex was measured at 760 nm using a Shimadzu UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan). Methanol was used as the blank and gallic acid used as standard. The used of gallic acid based on its stability and purity (Singleton and Rossi, 1965). The results were expressed as mg gallic acid equivalents (GAE)/g of sesame seed. Data were reported as means ± SD from 3 replications.

Statistical analysis

Results are presented as means value ± standard deviation. Statistical analysis between experimental results based on one-way ANOVA; pair-comparison of treatment means was achieved by Duncan's procedure at $P<0.05$ using statistical software SPSS 17 for Windows

Results and Discussion

The growth of L. plantarum *Dad 13, acid production and pH during fermentation of sesame milk*

The growth of *L. plantarum* Dad 13 in sesame milk during fermentation at 37°C is shown in Figure 1. *Lactobacillus plantarum* Dad 13 could grow in sesame milk fermentation. Viable cells increased from 7.24 log CFU mL^{-1} to 8.88 log CFU mL^{-1} after 18 h of incubation. This point out that carbon source in sesame milk was enough for *L. plantarum* Dad 13 growth. Sesame seed contained glucose (3.63%), fructose (3.43%), raffinose (0.59%), galactose (0.40%), stachyose (0.38%) and sucrose (0.17%) (Wankhede and Tharanatan, 1976). *Lactobacillus plantarum* can metabolized raffinose, fructose, galactose and sucrose to simple saccharide (Wheather, 1955). *Lactobacillus plantarum* Dad 13 can utilize carbohydrate in sesame milk as carbon source for its growth. Pangastuti *et al.* (2012) reported that *L. plantarum* Dad 13 could grow well in peanut milk without sugar addition.

The changes of titratable acidity and pH during sesame milk fermentation by *L. plantarum* Dad 13 are shown in Figure 1. The tritatable acidity increased from 0.06% to 0.24% during 18 h fermentation. The

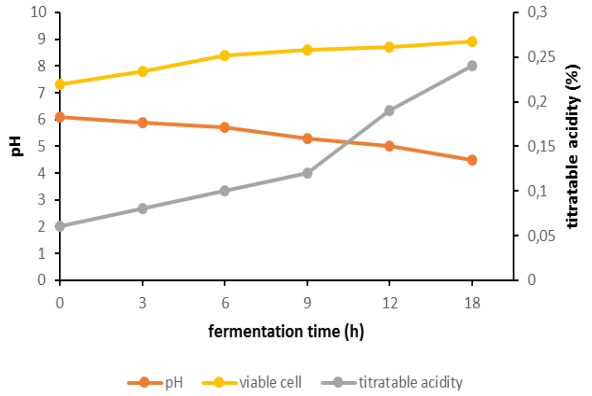

Figure 1. The growth, titratable acidity and pH during sesame milk fermentation by *L. plantarum* Dad 13 at 37°C

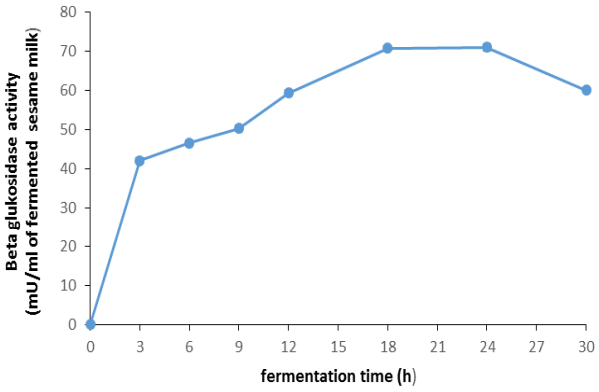

Figure 3. Beta glucosidase activity during sesame milk fermentation by *L. plantarum* Dad 13 at 37°C

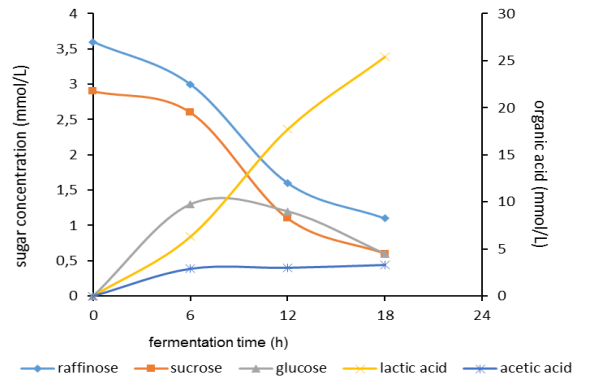

Figure 2. Sugar concentration and organic acid productions during sesame milk fermentation by *L. plantarum* Dad 13 at 37°C

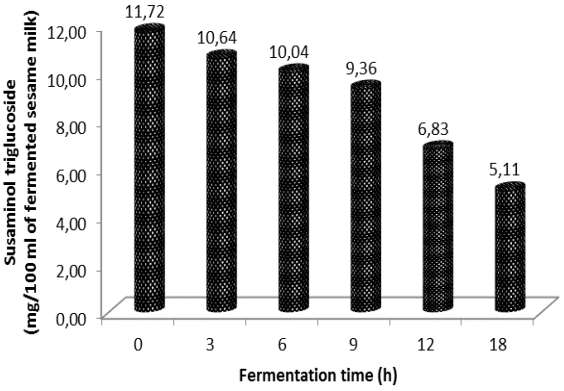

Figure 4. The change of sesaminol triglucoside during sesame milk fermentation by *L. plantarum* Dad 13 at 37°C

increasing of titratable acidity was in correlation with the cell population (Figure 1). The increase of the cell population results in a high metabolism rate, leads to the accumulation of lactic acid produced. Along with the lactic acid accumulation, the drop of sesame milk pH was occured. The pH was decreased from 6.1 to 4.5 during the 18 h fermentation. It means that *L. plantarum* Dad 13 could utilize nutrient in sesame milk for its growth and metabolism activity resulted in the production of acid and the decrease of pH. Pyo *et al.* (2005) showed that the increasing % titratable acidity and the decreasing pH were corresponded with the cell population. Fermentation of soymilk by *L. delbrueckii* 01181 (pH (4.4) and titratable acidity (1,12%)) contained cell population was higher than fermentation of soymilk by *L. plantarum* KFRI 00144 (pH (4,7) and titratable acidity (0.81%)). The increasing of titratable acidity along with the decreasing of pH was reported in another vegetable milk fermentation (Afaneh *et al.*, 2011; Pangastuti *et al.*, 2012).

Sugar consentration and organic acids production in fermented sesame milk

The sugar consentration and organic acids concentration in sesame milk fermentation at 37°C are shown in Figure 2. In this study, raffinose and sucrose changed during fermentation, concentration of raffinose decreased 69.4% (from 3.6 to 1.1 mmol/L) and concentration of sucrose decreased 79.3% (from 2.9 to 0.6 mmol/L) respectively. The raffinose was metabolized by *L. plantarum* Dad 13 into simple saccharides, such as sucrose, glucose, fructose and galactose as an energy source either for growth or metabolism via glycolysis pathway (Embden-Meyerhof pathway). This pathway represent the major means of glucose catabolism in most cells. Silvestroni *et al.* (2002) reported that *L. plantarum* was able to produce α-galactosidase enzyme when there are enough raffinose.

The glucose concentration increased and then decreased during fermentation. It was in correlation with the growth of *L. plantarum* Dad 13. *Lactobacillus plantarum* Dad 13 synthesized β-glucosidase to hydrolyze sesaminol triglucoside resulted in sesaminol aglycone and glucose. However,

Table 1. Radical scavenging activity and total phenolic content of fermented sesame milk

Fermentation time (h)	Radical scavenging activity (%)	Total phenolic content (mg GAE/g of sesame seed)
0	17.95 ± 0.64 a	3.80 ± 0.08 a
3	20.58 ± 0.37 b	4.94 ± 0.04 b
6	21.69 ± 0.64 c	5.22 ± 0.18 b
9	28.71 ± 0.64 d	5.99 ± 0.06 c
12	39.59 ± 0.98 e	6.25 ± 0.14 c
18	45.72 ± 0.64 f	8.01 ± 0.06 d

Different notation in the same coloumn indicated significant difference($P<0.05$)

the increase of viable cells need more glucose source for its growth, then glucose concentration decreased after 12 h fermentation. It means, the concentration of glucose depend on the hydrolysis rate of sesaminol triglucoside and the using of glucose source for its growth by *L. plantarum* Dad 13. Fructose was scarcely detected, which suggests that they were fastly consumed by the microorganisms during growth. *Lactobacillus plantarum* Dad 13 is a heterofermentative lactic acid bacteria which tends to use fructose rather than glucose as a terminal electron aceptor.

High metabolism rate of sugar, leads to the accumulation of organic acid produced. *Lactobacillus plantarum* Dad 13 produced mostly lactic acid and less amount of acetic acid in sesame milk. At the end of fermentation (18 h) 24.4 mmol/L of lactic acid and 3.33 mmol/L of acetic acid were produced. This result is in agreement with Coda *et al*. (2009), which showed that after fermentation, the concentration of lactic acid in yoghurt-like beverages made of mixture of cereals, soy and grape was varied from 20.5 to 33.8 mmol/L.

Beta-glucosidase activity in fermented sesame milk

The carbon source for the growth of *L. plantarum* Dad 13 in sesame milk was resulted of rafinose and sucrose content in sesame seed. Another carbon source was available in the form of sesaminol triglucoside, which is hydrolyzed by the β-glucosidase activity, resulted in sesaminol aglycone and glucose. Beta-glucosidase activity of *L plantarum* Dad 13 during sesame milk fermentation is shown in Figure 3. The activity of β-glucosidase increased during 24 h fermentation, followed by the decrease of sesaminol triglucoside content (Figure 4). This phenomenon indicated the ability of *L. plantarum* Dad 13 in utilizing sesaminol triglucoside as a carbon source by hydrolysis resulted in aglycone sesaminol and glucose.

During incubation, *L. plantarum* Dad 13 showed the highest enzyme activity at 18-24 h (70.8±0.56 – 71.06±0.9 mU/mL fermented sesame milk). During that incubation periode, the enzyme activity was not different statistically. It may be related to the growth characteristic of *L. plantarum* Dad 13 (Figure 1) which also shown the highest viability at 18 h incubation. This result was in aggrement with Tsangalist *et al.* (2002) which reported that β-glucosidase activity of *Bifidobacteria* has a positive correlation with the growth characteristic in soymilk fermentation. Moreover, Pyo *et al.* (2002) also pointed out that *L. delbrueckii* subs. *lactis* KFRI 01181 and *L. plantarum* KFRI 00144 showed the highest β-glucosidase activity at 24 h in soymilk fermentation, which also contained the most viable cell in fermented soymilk and then decreased. The level of β-glucosidase activity by *Bifidobacterium* varies significantly depends on types of growth medium (Choi *et al*., 1996). Tsangalis *et al.* (2002) also observed MRS-glucose broth as the most effective medium for the growth of *Bifidobacteria* for the β-glucosidase production.

Sesaminol triglucoside concentration in fermented sesame milk

The change of sesaminol triglucoside concentration during fermentation in sesame milk using *Lactobacillus plantarum* Dad 13 is shown in Figure 4. The concentration of sesaminol triglucoside before fermentation was 11.72 mg/100 mL of sesame milk (130.27 mg/100g sesame seed). Moazzami *et al*. (2006) reported that analysis of 65 different varieties of sesame seed indicated that the content of sesaminol triglucoside ranged from 36 to 1560 mg/100 g of sesame seed. At the end of sesame milk fermentation, sesaminol triglucoside concentration decreased to 5.11 mg/100 mL of sesame milk. The decrease of sesaminol triglucoside concentration was in correlation with the β-glucosidase activity which showed an increase during sesame milk fermentation (Figure 3). The reduction of sesaminol triglucoside may be based on the hydrolytic reaction catalyzed by β-glucosidase produced by *L. plantarum* Dad 13. Glucose, as a product of sesaminol triglucoside hydrolysis, was detected during fermentation of sesame milk (Figure 2). These results pointed out that *L. plantarum* Dad 13 was able to transform sesaminol glucosides to aglycone. Fukuda *et al.* (1985) showed that the results of aqueous ethanol hydrolysis extracts from sesame oil cake with β-glucosidase indicated those lignans type antioxidant are present both as free phenolic compounds and as aglycone moieties

of glycosides in sesame seed.

Antioxidant activity and total phenolic content in fermented sesame milk

The antioxidant activity and total phenolic content in fermented sesame milk using *Lactobacillus plantarum* Dad 13 are shown in Table 1. Fermentation of sesame milk with *L. plantarum* Dad 13 gave an enhance radical scavenging activity by 2.55 times at the end of fermentation compared to the initial time (Tabel 1).

Beta-glucosidase enzyme produced by *L. plantarum* Dad 13 appeared to hydrolyze β-1,2-glycoside bond between glucose and lignan molecules in sesame milk fermentation. The free form lignans is more reactive, therefore it showed higher activity in reducing phosphotungstat and phosphomolibdenum in Folin-Ciocalteu reagent, increasing total phenolic content in sesame milk fermentation. The increasing radical scavenging activity during fermentation was followed by the total phenolic content increased (Table 1). These results suggested that the hydrolysis of sesaminol triglucoside into sesaminol aglycones contributed to increase antioxidant activity in the fermented sesame milk performed by the fermented sesame milk. According to Fukuda *et al.* (1985) the addition of hydroxyl group on the atom C-2 of sesaminol may responsible for the increase of antioxidant activity.

Conclusion

Lactobacillus plantarum Dad 13 grew well in sesame milk, indicated the ability of *L. plantarum* Dad 13 of utilizing carbohydrate in sesame milk as a carbon source. This ability was supported by the synthesis of β-glucosidase enzyme to hydrolyze sesaminol triglucoside to sesaminol aglycone and glucose. The release of active aglycone sesaminol resulted in the increase of antioxidant activity and total phenolic content. The ability of *L. plantarum* Dad 13 to hydrolyze the β-glucoside lignans in sesame milk may have nutritional benefits to the bioavaibility of lignans. These results need further study for application of *L. plantarum* Dad 13 to produce a sesame-based functional fermented product.

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