Chemical and Sensory Quality of Milk Fermented by Starter Combination of *Lactobacillus plantarum* Dad 13, *Lactococcus lactis*, and Yeast

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ABSTRACT

Lactobacillus plantarum Dad 13 is a typical of bacteria from "Dadih" (traditional fermented milk from West Sumatra, Indonesia). The purpose of this study was to evaluate the effect of starter combination of *Lactobacillus plantarum Dad* 13, *Lactococcus lactis* and yeast (*Saccharomyces cerevisiae* and *Kluyveromyces marxianus*) on the chemical and sensory quality of fermented milk. The nine combinations of starter to produce fermented milk were: 1) *L. plantarum* Dad 13, 2) *L. plantarum* Dad 13 + *L.lactis*, 3) *L. plantarum* Dad 13 + *S. cerevisiae*, 4) *L. plantarum* Dad 13 + *K. marxianus*, 5) *L. plantarum* Dad 13 + *L. lactis* + *S. cerevisiae*, 6) *L. plantarum* Dad 13 + *L.lactis* + *K. marxianus*, 7) *L.lactis*, 8) *L. lactis* + *S. cerevisiae*, 9) *L. lactis* + *K. marxianus*. The evaluated chemical quality of fermented milk included total solid, fat and protein, whereas the sensory quality included taste, aroma, texture and hedonic test. The results showed that combination of starter had no effect on total solid, fat, and protein of fermented milk. However, the combination of starter could affect on taste, aroma, texture and hedonic test of fermented milk ($p<0.05$). Fermented milk from the a single starter of *L. plantarum* Dad 13 had the lowest score ($p<0.05$) for the taste, aroma, texture and hedonic test, while other product from the combination of *L. Plantarum* Dad 13 + *L. lactis* + *K. marxianus* had highest score in taste ($p<0.05$). In conclusion, the combination of starter had no adverse effect on the chemical quality but may affect on the sensory quality of fermented milk.

Keywords: Fermented milk, Chemical quality, Sensory quality, Starter combination.

INTRODUCTION

The traditional Dadih has no standard of manufacture, so the quality of the products produced varies widely. Traditionally, Dadih is made by putting buffalo milk in a bamboo tube and covered with banana leaf and then incubate during 2 to 3 days. There is a desire to produce fermented products with uniform product quality and taste that consumers love. Types of microbial starter, temperature, fermentation time and raw materials used may affect the chemical composition and flavor of fermented milk.

The utilization of more than one kind of starter in the manufacture of fermented milk allows lactic acid bacteria (LAB) synergize to form a symbiosis. An interaction between starter culture has many benefits, one of which is increasing the aroma component (Jakobsen and Narvhus, 1996). *Lactobacillus plantarum* is a facultative heterofermentative bacteria (Holzaptel and Wood, 2014), able producing lactic acid and also acetic acid, ethanol, and CO_2 (Hutkins, 2006). *Lactococcus lactis* is a homofermentative bacteria (Hutkins, 2006) and able to produce 90% lactate from the total final metabolic product produced (Papagianni, 2012). The interaction between yeast and lactic acid bacteria may affect the characteristics, and quality of the fermentation product primarily leads to the profile of the compounds that are essential for the improvement of organoleptic qualities (Narvhus, 2003). The specific interaction forms between yeast and lactic acid bacteria depend on yeast and bacteria (Jakobsen and Narvhus, 1996; Viljoen, 2006). During fermentation, some yeast can produce organic acids derived from lactose metabolism and also produces CO_2 and alcohol (Tamime and Robinson, 2007).

In this study, fermented milk was prepared using a combination of starter, utilize LAB (*L. plantarum* Dad 13, *L. lactis*), and yeast (*Kluyveromyces marxianus* or *Saccharomyces cerevisiae*). *L. plantarum* is the dominant majority of BAL found in the Dadih (Sunarlim, 2009). The utilization of *L. plantarum* Dad 13 derived from Dadih combined with *L. lactis* and yeast (*K. marxianus* or *S. cerevisiae*) is expected to improve the chemical and organoleptic characteristics of fermented milk.

MATERIALS AND METHODS

Starter preparation. As much as one inoculating loop each bacterial culture *L. plantarum* Dad 13 and *L. lactis* were transferred into a 10 ml tube containing MRS medium which had been sterilized by autoclave at 121°C, 15 psi for 15 minutes. The LAB were inoculated into MRS medium, then incubated at 37°C (*L. plantarum* Dad 13) and 30ºC (*L. lactis*) for 24 hours. The culture of yeast (*S. cerevisiae* FNCC 3012 and *K. marxianus* FNCC 3011) were transferred into a 10 ml tube containing YPG medium sterilized by autoclave at 121°C, 15 psi for 15 minutes. Yeast inoculated into the YPG medium, then incubated at 30°C for 24 hours. The starter preparation was performed by inoculation 10%(v/v) of inoculum into 100 ml of 18% (w/v) skim milk which had been sterilized at 110°C, 13 psi for 10 min, then incubated for 12-18 h to form a curd . The result is called mother starter. As much as of 10% mother starter inoculated into 100 ml of 18% (w/v) sterilized skim milk followed by incubation for 12-18 hours to form bulk starter. The starter inoculated into milk to perform fermentation or when not immediately used could be stored at 10°C.

Fermented milk. Fresh cow milk was pasteurized at 85°C for 30 minutes, then cooled to 40°C. A total of 3% (v/v) starter was then inoculated into milk followed by incubation at 37°C for 18 hours. The comparison of starter used is shown in Table 1.

Table 1. Combination of Starter and Starter Ratio

Combination of Starter	Code	Ratio
L. plantarum Dad 13	K1	-
L. plantarum Dad 13 + *L. lactis*	K2	1 : 1
L. plantarum Dad 13 + *S. cerevisiae*	K3	2 : 1
L. plantarum Dad 13 + *K. Marxianus*	K4	2 : 1
L. plantarum Dad 13 + *L. lactis* + *S. cerevisiae*	K5	1 : 1 : 1
L. plantarum Dad 13 + *L. lactis* + *K. Marxianus*	K6	1 : 1 : 1
L. lactis	K7	-
L. lactis + *S. cerevisiae*	K8	2 : 1
L. lactis + *K. Marxianus*	K9	2 : 1

Water content. Moisture content test refered to the method conducted by Sudarmadji et al. (2007). One gram of sample was placed in a porcelain cup previously heated at 105°C for 24 hours then cooled in the desiccator for an hour and weighed. The sample was then dried in the oven at 105°C for 12 hours then cooled in the desiccator for and hour and weighed.

$$\text{Water content} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Weight of sample}} \times 100\%$$

Lactose Content. Lactose content was determined using method conducted by Sudarmadji et al. (2007). The amount of 25 ml of fermented milk was fed into a 50 mL flask, add 5 ml of $ZnSO_4$ reagents and shaked vigorously. An amount of 5 ml NaOH solution (0.75 N) added to the flask and then shaked vigorously. The sample was then diluted with distilled water until marks. The suspension was kept for about 10 minutes to precipitate all the proteins. Suspension filtered with filter paper, and filtrate taken. The volume of the filtrate was calculated theoretically by reducing the volume of the precipitated protein (density 1.25) and the volume of fat (density 0.9) from the initial volume of 50 mL. Clear filtrate was taken as much as 5 mL, then put into 250 mL Erlenmeyer. A total of 20 mL of distilled water and 20 mL of 10% KI solution were added, then 50 mL of Chloramine-T solution was added. The solution was then shaken vigorously and allowed to stand for 90 minutes. A total of 10 ml of 2 N HCl solution was added to the solution, then titrated with $Na_2S_2O_3$ solution until pale yellow. The starch solution indicator was then added and the titration continued until the color was gray. The blank solution was prepared by replacing 25 ml of fermented milk with 25 ml of distilled water. The blank solution was titrated as in the sample solution. Lactose content was calculated by the formula:

$$A = (T_B - T_S) \times N \times 0.171 \times \frac{100}{5}$$

$$\text{Lactose content} = A \times \frac{B}{100} \times \frac{100}{\text{mL of sample}}$$

where:

A = Lactose in filtrate (g/100 mL of filtrate)

T_S = Sample titration

T_B = Blank titration

N = Normality of $Na_2S_2O_3$

B = Volume of filtrate in 50 mL of dilution

Lipid Content. Measurement of lipid content in fermented milk was performed using the Babcock method. The amount of 18 grams of sample was weighed into a Babcock tube (1: 1 dilution), then added as much as 17.5 ml of H_2SO_4 (to destruct protein and damage lipid emulsions). The sample was centrifuged for 5 minutes, added 60°C of distilled water up to the neck of the Babcock tube. The sample was then centrifuged for 2 minutes. The lipid reading result was determined by reading the scale on the neck of the Babcock tube (Sudarmadji et al., 2007).

Protein Content. Determination of protein content was investigated using Kjeldahl method whose stages consist of destruction, distillation, and titration. A total of 2 g samples was placed inside the destruction tube. Each tube added 12 mL of concentrated H_2SO_4 and Kjeldahl tablets (consisting of sodium sulfate, copper (II) sulfate, selenium, the polymer of ethylene glycol). The sample was then destructed at 420°C for an approximately one hour using Kjealtec 2200 Auto Destruction Unit tool until the clear solution obtained. The sample was subsequently cooled and distilled with Kjealtec 2200 Auto Distillation Unit by adding 50 mL of 40% NaOH and 80 mL of distilled water into the tube. During the distillation, the hot vapor formed would enter the tube. The formed nitrogen would condense into an Erlenmeyer flask containing 30 mL of a solution consisting of 4% H_3BO_3, a methylene red indicator and a BCG indicator (Bromo Cresol Green). At the end of the process, 150 mL of distillate was obtained. The distillate was then titrated with 0.1 N HCl until indicator color changed. Protein content was calculated using the formula

$$\%N = \frac{\left(\text{ml HCl}_{\text{sample}} - \text{ml HCl}_{\text{blank}}\right) \times 14,008 \times N_{\text{HCl}}}{\text{Weight of sample}} \times 100$$

$$\%\text{Protein} = \%N \times 6,38$$

Sensory Tests. Sensory tests were performed based on the hedonic test. The data were analyzed by variance analysis (ANOVA) and continued by DMRT (Duncan Multiple Range

Test). Panelists were required to provide product judgment according to the panelist's preference with the score. Parameters in the test included aroma, sour taste, and appearance.

Statistic. The result of data tabulation in normality test of data using Shapiro-Wilk test. Analysis of the effect of treatment was done by One Way ANOVA test. The data obtained was processed using IBM SPSS 22.

RESULTS AND DISCUSSION

Chemical Quality. The chemical quality of fermented milk (water, lactose, lipid, and protein contents) is shown in Table 2. Water content is one of the most important characteristics of food. Moisture content can affect the texture and flavor of the food (Zurriayanti et al., 2011). Statistical analysis showed that the combination of starter treatment on fermented milk production did not affect the fermented milk content. Sunarlim et al. (2007) also observed that there was no influence of starter combination on water content. Previous studies have reported that the moisture content in Dadih ranged from 69.91 to 75.23% (Afriani et al., 2012) while in Kefir about 87.5% (Otles and Lagindi, 2003). The relatively similar fermented milk content for each starter combination was due to the similarity of raw materials and the added starter concentration (3%). Besides, water was not synthesized by bacteria during fermentation and only acts as a microorganism growth medium so that it affected its growth rate.

Lactose is a major carbohydrate component in milk and will decompose into lactic acid during fermentation (Farnworth, 2008). Levels of lactose in raw materials (fresh milk) in this study about 4.9% and experienced a decrease during fermentation. The lowest lactose content of 3.58% was obtained from a combination of starter *L. plantarum* Dad 13 + *L. lactis* + *K. marxianus*. It could be observed that fermented milk from the combination of *K. marxianus* tends to have lower lactose levels that show its ability to metabolize lactose. The decrease in lactose in fermented milk was consistent with the increase in lactic acid and also the decline in pH. Manan et al. (1999) reported lactose levels of 3.63% in Dadih, whereas according to Usmiati (2007), lactose levels in kefir were 4.5%. During fermentation, BAL converts 20% to 30% of lactose to lactic acid (Chandan et al., 2006). Differences in lactose concentration in fermented milk could be caused by various factors, including lactose content of raw materials, type of starter used and incubation time.

Table 2 shows that the combination of starter for raw materials and the same starter concentration (3%) did not significantly affect the protein content of fermented milk. This result was in accordance to Nakazawa and Hosono (1992) who observed no change in the physiological value of cow's milk protein by lactic acid fermentation. Taufik (2004) and Afriani (2012) also observed no effect of LAB type on protein content in cow milk Dadih. Protein in fermented milk is the total amount of milk protein of raw materials used by LAB for the fermentation process (Yusmarini and Efendi, 2004). Lactic acid bacteria is a type of bacteria that requires complete nutrition for life including amino acids and vitamins. However, LAB is not able to synthesize various amino acids or vitamins in the cell. Because LAB does not have a system or mechanism that regulates the biosynthesis of amino acids, LAB will degrade the milk casein as a major source of amino acids and peptides (Widodo, 2003). Kefir contains a protein of 3.5% (Usmiati, 2007), and ranged from 3.76-4.79% on Dadih (Afriani et al., 2012). The protein content in fermented milk in this study meets the standard of Codex 243-2003 which was 2.7% (Codex, 2011).

Table 2. Chemical Quality of Fermented Milk form Different Starter Combination

Starter Combination	Water Content, %	Lactose Content, %	Protein Content, %	Lipid Content, %
K1	80.17 ± 0.48	4.18 ± 0.05^{c}	3.09 ± 0.07	3.08 ± 0.03
K2	80.15 ± 0.37	3.94 ± 0.14^{abc}	3.13 ± 0.02	3.07 ± 0.08
K3	80.13 ± 0.87	4.00 ± 0.35^{bc}	3.12 ± 0.06	3.09 ± 0.06
K4	80.14 ± 0.54	3.98 ± 0.32^{bc}	3.07 ± 0.16	3.10 ± 0.02
K5	80.15 ± 0.34	3.72 ± 0.10^{ab}	3.12 ± 0.03	3.07 ± 0.10
K6	80.12 ± 0.07	3.58 ± 0.26^{a}	3.11 ± 0.05	3.11 ± 0.08
K7	80.15 ± 0.80	4.10 ± 0.02^{c}	3.10 ± 0.08	3.09 ± 0.05
K8	80.13 ± 0.53	3.86 ± 0.03^{abc}	3.10 ± 0.03	3.08 ± 0.07
K9	80.15 ± 0.36	3.72 ± 0.14^{ab}	3.13 ± 0.05	3.07 ± 0.07

K1: *L. plantarum* Dad 13, K2: *L. plantarum* Dad 13 + *L. lactis*, K3: *L. plantarum* Dad 13 + *S. cerevisiae*, K4: *L. plantarum* Dad 13 + *K. marxianus*, K5: *L. plantarum* Dad 13 + *L. lactis* + *S. cerevisiae*, K6: *L. plantarum* Dad 13 + *L. lactis* + *K. marxianus*, K7: *L. lactis*, K8: *L. lactis* + *S. cerevisiae*, K9: *L. lactis* + *K. marxianus*. $^{a-c}$Values with different superscripts are significantly different in a column ($p<0.05$).

During fermentation, there is a decrease in milk fat levels due to microbial lipolytic activity. Lactic acid bacteria have secondary lipolytic activity, which occurs after other microorganisms break down milk fatty acids into simpler compounds (Bottazi, 1983). Tamime and Robinson (2007) stated that the hydrolysis of fat by starter cultures occurs only at insufficient levels. The fat content of the research raw material (fresh milk) was 3.19 and decreased in the average range from 3.07 to 3.11 (Table 2). The ANOVA assay showed no effect of the starter combination used in the fermented milk fat content and did not show a regular pattern. The level of fat in Kefir was 3.5% (Otles and Lagindi, 2003), while Dadih contains 10.50 to 12.06% lipid content (Sisriyenni and Zurriyati, 2004). The standard fat content of fermented milk and kefir according to Codex (2011) was less than 10%. Thus the fat content of fermented dairy products produced in this study met Codex 243-2003 standard.

Organoleptic test. Organoleptic testing aimed to determine the acceptance of consumers. The results of the organoleptic test is shown in Table 3. Criteria for the taste test (acidity) include: 1) Very not acid, 2) not acid, 3) slightly acid, 4) acid, 5) very acid. The result of ANOVA test showed that starter combination had a significant effect on acidity level ($p<0.05$). Overall fermented milk had a sour taste except made from a single starter *L. plantarum* Dad 13. Fermented milk made from single starter *L. plantarum* Dad 13 was considered slightly acid (value 3.10 ± 0.08). Acidity levels are affected by lactic acid levels in fermented milk (Tamime, 2005). The combination of LAB starter is also reported to increase the rate of lactic acid production (Tamime and Robinson, 2007).

Table 3. Organoleptic Test Results of Fermented Milk on Different Starter Combinations

Starter Combination	Taste	Aroma	Texture	Acceptance
K1	3.07 ± 0.27^{a}	2.69 ± 0.48^{a}	2.30 ± 0.48^{a}	1.92 ± 0.49^{a}
K2	4.30 ± 0.48^{bcd}	4.07 ± 0.27^{b}	4.00 ± 0.00^{b}	1.15 ± 0.37^{b}
K3	4.00 ± 0.00^{b}	4.00 ± 0.00^{b}	4.00 ± 0.00^{b}	1.07 ± 0.27^{b}
K4	4.00 ± 0.40^{b}	4.00 ± 0.00^{b}	4.00 ± 0.00^{b}	1.00 ± 0.00^{b}
K5	4.38 ± 0.50^{d}	4.15 ± 0.37^{b}	4.00 ± 0.00^{b}	1.00 ± 0.00^{b}
K6	4.53 ± 0.15^{d}	4.15 ± 0.10^{b}	4.00 ± 0.00^{b}	1.00 ± 0.00^{b}
K7	4.00 ± 0.10^{b}	4.00 ± 0.37^{b}	4.00 ± 0.00^{b}	1.00 ± 0.00^{b}
K8	4.15 ± 0.37^{bc}	4.15 ± 0.55^{b}	4.00 ± 0.00^{b}	1.15 ± 0.15^{b}
K9	4.30 ± 0.48^{bcd}	4.15 ± 0.53^{b}	4.00 ± 0.00^{b}	1.15 ± 0.15^{b}

K1: *L. plantarum* Dad 13, K2: *L. plantarum* Dad 13 + *L. lactis*, K3: *L. plantarum* Dad 13 + *S. cerevisiae*, K4: *L. plantarum* Dad 13 + *K. marxianus*, K5: *L. plantarum* Dad 13 + *L. lactis* + *S. cerevisiae*, K6: *L. plantarum* Dad 13 + *L. lactis* + *K. marxianus*, K7: *L. lactis*, K8: *L. lactis* + *S. cerevisiae*, K9: *L. lactis* + *K. marxianus*. $^{a\text{-}c}$Values with different superscripts are significantly different in a column ($p<0.05$).

The aroma test aimed to know the level of consumer preference to the aroma. Criteria assessment includes 1) very dislike 2) do not like 3) rather like 4) likes 5) very like. ANOVA test results showed that fermented milk made from single starter *L. plantarum* Dad 13 had the lowest score and significantly different ($p<0,05$) to other product. Yusmarini and Efendi (2004) observed that the fermentation process could reduce the typical aroma of milk due to organic acids formed during the fermentation process to enhance the flavor. Jakobsen and Narvhus (1996) showed that interaction between starter culture could increase the formation of aroma components.

Favorite criteria for fermented milk texture included 1) very likes 2) dislikes 3) rather likes 4) likes 5) and very fond. The ANOVA test on the fermented milk texture criterion showed that fermented milk made from single starter *L. plantarum* Dad 13 had the lowest and significantly different ($p<0.05$) score compared to the products derived from other combinations. Fermented milk made from single starter *L. plantarum* Dad 13 had a less dense texture compared to other fermented milk, so it was less favorable to the panelists. Panelists prefer a thick, creamy-looking texture. The process of milk fermentation causes the formation of the acidic atmosphere (low pH) this is due to disruption of balance casein at the isoelectric point (pH = 4.6). Casein will then coagulate to form a coagulant. Under these conditions, the milk casein is negatively charged while the lactic acid molecule during the fermentation process is positively charged. The intersection between casein and lactic acid causes a neutralization process, so the casein settles. This affects the texture of fermented milk.

Criteria for acceptance of fermented milk products included 1) receiving, 2) neutral, 3) refusing. The result of ANOVA test on the acceptability of fermented milk products stated that fermented milk using single starter *L. plantarum* Dad 13 was significantly different ($p<0.05$) compared with the products of other combinations. Panelists gave a neutral assessment of fermented milk acceptability made from single starter *L. plantarum* Dad 13, while fermented milk with other starter combinations was well received. Sunarlim et al. (2007) in his study found that fermented milk made from single starter *L. plantarum* was less favorable than fermented milk with three combinations of *L. plantarum* with *L. bulgaricus* and *Streptococcus thermophilus*.

CONCLUSIONS

The combination of starter had no adverse effect on the chemical quality but may affect on the sensory quality of fermented milk.

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