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Full Length Research Paper

The effect of dual fortifications of fe and zn in synbiotic fermented milk (*Lactobacillus plantarum* Dad13-fructooligosaccharide) on the levels of acidity (pH) and the total of lactic acid bacteria (LAB) during storage

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Abstract

To analyze the influence of dual fortifications of fe and zn in synbiotic fermented milk on the levels of acidity (pH) and the total of lactic acid bacteria (LAB) during the storage periode. Synbiotic fermented milk was made from local isolate *Lactobacillus plantarum* Dad 13-Fructooligosaccharide, then classified into 3 groups, namely: 1) no fortification (NF), 2) fortified with NaFeEDTA and zinc acetate (NZ), and 3) fortified with fe gluconate and zinc acetate (FZ). The ratio of fe and zn was 100 ppm: 50 ppm. pH value of those three kinds of synbiotic fermented milk increased in the first week and tended to decrease in the final week of storage but not significantly different (p > 0.05). There was no significant difference of total LAB (p > 0.05) during storage. Fortification of fe and zn did not affect both pH and LAB of synbiotic fermented milk during periods of four-week storage. Dual fortifications of fe and zn can be applied in synbiotic fermented milk, as a potential way to combat anemia.

Keywords: Fermented food, prebiotics, probiotics, symbiosis, lacic acid bac.

INTRODUCTION

Iron deficiency is one form of the malnutrition that is quite considerable in the world. This deficiency can cause various kinds of disease, one of them is anemia. At 2007, Basic Health Survey (Riskesdas) indicated that the national prevalence of anemia reached 11.9%, 12.8 % of them experienced by the children. Furthermore, based on Household Health Survey (2001), prevalence of iron deficiency anemia (IDA) in toddler aged 4-5 years and as much as 32.1% of school-age children (5-19 years) as much as 26.5%. The most severe effect of anemia is an increasing number of deaths in mothers and children. In addition, anemia also has an impact on cognitive and physical development in children (Watkins *et al.,* 1998; WHO, 2008). In adults, anemia causes a decreased in work productivity. Anemia is not only caused by a lack of nutrients in the form of fe but also zn (Whittaker, 1998). Zinc has role in celular replication, DNA, RNA, and protein synthesis (Seleet *et al.,* 2011).

Numerous attempts have been reached in order to handle anemia, such as iron supplementation and fortification. Food fortification with micronutrients is one of the main strategies capable ofimproving the micronutrient status of food (Siagian, 2003) and combat anemia (Baltussen *et al.,* 2004). Contradictively, many studies showed the negative effects of iron supplementation. Study in Cote de Ivory showed that enterobacteria increases in the iron fortification group while lactobacilli decreases (Zimmermann 2010). Iron supplementation can induce the rapid growth of gut pathogenic bacteria. Pathogens bacteria need iron for their growth (Andrews *et al.,* 2003; Naikare *et al.,* 2006).

Probiotics improve both cellular and humoral immunity (Sazawal *et al.,* 2004). Some indigenous probiotics has been isolated from Indonesian local food. One of them is *Lactobacillus plantarum* Dad13 that is isolated from "dadih', the Indonesian traditional food made from buffalo's milk. These bacteria showed humoral immune effects (Kusumawati 2006). *In vitro*, *L. plantarum* Dad 13 showed extracellular anti microbial activity on Salmonella. Prebiotics can support the growth of probiotics by provide selective substrates for its growth (Gibson *et al.* 2004). Prebiotics fermentation by LAB can decrease pH (Rowland *et al.,* 1997) so that mineral absorption can increase (Rastall, 2010).

Food carriers that are most suitable for fortification of prebiotics and probiotics is fermented milk. Besides being easily digested, fermented milk can minimize lactose intolerance. Milk is a good growth medium for microorganisms but it is easily damaged, so it needed a better processing. One common trait possessed by fermented milk is its sour taste and have a short shelf life (Gianti and Evanuarini, 2011). Therefore, the storage of fermented milk must be considered, because the storage will affect the total bacteria, LAB levels, and quality of fermented milk. Foods that contain acid can usually last a long time. Total LAB and acidity will affect the quality of the product and its impact is public acceptability. This study is expected to provide a solution to overcome the problems of malnutrition.

MATERIALS AND METHODS

Synbiotic fermented milk

The research design is a simple randomized design with two treatments (one control and one fortification). Synbiotic fermented milk was made from *Lactobacillus plantarum* Dad 13-Fructooligosaccharide, then classified into 3 groups, namely: 1) no fortification (NF), 2) withNaFeEDTA and zinc acetate fortification (NZ), and 3) with fe gluconate and zinc acetate fortification (FZ). The ratio of fe and zn was 100 ppm: 50 ppm.

Instruments

The equipment used in this study include measuring cup tool, OSE, aluminum foil, rubber, plastic, analytical balance, laminar space, waterbath, mixer, refrigerator, incubator, thermometer, pH meter, glass, pint flask, funnel glass, glass and cup sour milk, tubes erlenmeyer 1000 ml, 250 ml (Pyrex) glass bottles and erlenmeyer and autoklave for sinbiotic fermented milk maker. BAL measurements using petri dish, laminar air flow, 37^0C incubator, water bath, Quebec Colony Counter, cold room 4^0C, vortex, glassware, conicel, bluetip, test tube rack, 1 ml micropipette. Testing the level of acidity or pH value using a vortex homogenization tools and measuring instruments such as pH meters (pH Eutech 510 instrument).

The fortification of the iron used was NaFeEDTA (Akzonobel, Ferrazone), Fe gluconate from Merck ® Germany, and fortificant zinc used was Zn acetate (Merck). Other ingredients such as probiotic L. *plantarum* Dad 13 from *FNCC (Food and Nutrition Culture Collection)* Food and Nutrition Study Center of Universitas Gadjah Mada, Yogyakarta, Lactona Instant skim milk (PT. Mirota Indonesia) and sucrose sukrosa (Gulaku white sugar), prebiotic fructooligosaccharides (orafti) and Aquades. For the calculation of the amount of LAB, the ingredients used, among others, MRS agar (de Mann ROGOSA Sharpe), synbiotic fermented milk, aquades, and NaCl.

Fermented milk making process

Skim milk, FOS, sugar and fortificant were dissolved into desirable volume of water. Fortified milk was pasteurized at 80^oC for 30 minutes. Then, 1% starter of *L.plantarum* Dad 13 was inoculated and incubated at 37^o c for 24 hours.

Analysis

Milk was stored and measured for pH and total LAB for four weeks. pH was measured with pHmeter while total LAB was counted with media selective agar (MRS agar).

Statistical analysis

Data were analyzed using the *Kruskal-Wallis* test to determine differences between groups. The results data were considered significant if the results are $p < 0.05$.

RESULTS

pH

Time measurement started from week 0 day, thatis one day after the fermentation process took place, followed by the 1^{st}, 2^{nd}, 3^{rd} week. The last period of storage was one month or the last day of 4^{th}week. Milk stored in temperature of 4^0C. The measurement of pH was performed in three repetitions at each type of milk, and subsequently the results of the measurements were averaged. The results analysis of the degree of acidity (pH) of the three types of milk during the storage period of four weeks is shown in Table 1.

The results of *Kruskal Wallis* test showed there was no significant difference of the three types of fermented milk neither from NZ, FZ nor N ($p>0.05$). Based on the

Table 1. The Degree of acidity (pH) of there types of fermented milk

	NZ	FZ	NF	p
0 Week	4.32 ± 0.06	4.34 ± 0.01	4.24 ± 0.16	0.733
1st Week	4.50 ± 0.09	4.47 ± 0.01	4.46 ± 0.01	0.491
2nd Week	4.40 ± 0.01	4.40 ± 0.02	4.42 ± 0.00	0.164
3rd Week	4.36 ± 0.09	4.41 ± 0.00	4.36 ± 0.00	0.295
4th Week	4.25 ± 0.02	4.20 ± 0.00	4.21 ± 0.01	0.056

Values expressed in χ ± SD
NZ : Synbiotic fermented milk with NaFeEDTA and zn acetate
FZ : Synbiotic fermented milk with fe gluconate and zn acetate
NF : Synbiotic fermented milk (without fortification)

Table 2. Total of Lactic Acid Bacteria (log CFU g^{-1})of there types of fermented milk

	NZ	FZ	NF
0 Week	9.7559	8.0253	9.3043
1st Week	7.7160	7.6434	7.9191
2nd Week	10.4771	9.7482	10.4771
3rd Week	8.6532	10.1492	8.4843
4th Week	6.3617	6.5682	8.9031

Values expressed in χ ± SD
NZ : Synbiotic fermented milk with NaFeEDTA and zn acetate
FZ : Synbiotic fermented milk with fe gluconate and zn acetate
NF : Synbiotic fermented milk (without fortification

results of average pH, from day 0, the highest pH was shown by FZ milk, followed by NZ, while pH control fermented milk has the lowest pH. The overall average pH of milk through the whole week ranged from 4.2-4.4. There was an increasing on pH levels of the three types of synbiotic fermented milk for a week of storage. pH then decreased until the last storage on week 4.

Lactid Acid Bacteria

LAB calculations converted in the form of log CFU g^{-1} and being averaged over in each group. The average result of each group are shown in table 2. The total LAB of the three types of milk were highest in the 2^{nd} week of storage. However, there are several types of milk which have a total LAB which are unstable, for example, NZ in week 1 has decreased, then tend to rise again at week 2, and further decreased during 4 weeks of storage. This was also observed by fermented milk NF and NZ. Some things that can cause such deviations include aseptic treatment which caused the possibility of the contamination making LAB cannot grow well. Furthermore, the activity of opening and closing the samples that repeatedly performed each week also reduce the sterility of samples. From the results of *Kruskal Wallis*, it can be concluded that there is no significant difference of total LAB among the three groups of milk; neither from NZ, FZ, nor NF (p>0.05).

DISCUSSION

Based on the results of statistical tests, it is known that there were no significant differences in pH among three types of synbiotic fermented milk during storage. Average pH levels were measured in three types of synbiotic fermented milk through the shelf life of one month is in the range between 4.2 to 4.4.

Iron fortification did not really affect pH levels too significantly in the process of saving up to one month period. It also relates to the use of starter *L. plantarum* Dad 13 which its role is to maintain the stability of pH in the synbiotic fermented milk (Sari, 2011).

The levels of acidity (pH) of fermented milk products are affected by the increased production of lactic acid by lactic acid bacteria (LAB). To produce acid, LAB use carbohydrates like lactose that being overhauled so that the pH of the fermented milk being dropped. Lactose that found in milk is the only carbohydrate energy available as LAB for the formation of lactic acid by the enzyme lactate dehidrogenase (Balia *et al.,* 2011).

Based on the pH levels during storage period in a week, there was an increase in pH levels of the test results at week 0 to week 1 from the three synbiotic fermented milk. A total of LAB of three groups milk, the highest level was shown in the second week during the fermentation process. When compared with pH, the highest range of total bacterial was observed at pH 4.4.

Until the last week of the storage process, the figure shows that synbiotic fermented milk without fortification has higher total LAB than when compared with NZ and FZ milk.

One source of nutrients for the growth of pathogenic bacteria is iron substance (Naikare *et al*., 2006). Pathogenic bacteria also use an iron substance source by taking from its environment. Pathogenic bacteria have an increased ability to acquire iron from iron-binding proteins in the body, compared with the avirulent strain. Most of gram-negative bacteria such as *Salmonella*, *Shigella*, and some strains of *Escherichia coli* are pathogens, and iron acquisition plays an important role in virulence and colonization of most of pathogenic bacteria (Naikare *et al.,* 2006). However, some defense intestinal bacteria such as *Lactobacillus* play an important role in preventing colonization of enteric pathogens but do not require iron substances (Bezkorovainy *et al.,* 1996). Iron substance in fermented fortification milk allegedly used by pathogenic bacteria.

This study showed that fortification in fermentation milk does not affect the total LAB bacteria during storage process. LAB are good bacteria which are beneficial in the fermentation process and its influence in health human food (Harmayani *et al.,* 2001). The total LAB in fermented milk signify the lactose which being broken into lactic acid also increased, so the acidity of fermented milk is also increased. Taking into account the results of pH and LAB measurements during four weeks of storage, fermented milk are best consumed in the second week of storage and at the pH range of 4.4. At that time the LAB grow optimally.

Several weaknesses of this study is the lack testing to the pathogenic bacteria that it cannot be concluded that substances contained in synbiotic fermented milk are actually used by pathogenic bacteria. So there is a need for a comparative analysis of pathogenic bacteria on a weekly basis. The LAB calculation method used also has some weaknesses, namely the subjectivity of researchers. There are needs of another method to measure the LAB so that the results obtained can be precise and accurate.

The samples used should be placed in different Erlenmeyer every week so there is no need to use it anymore after being used. In this study, the same sample used repeatedly so many activities to open-closed Erlenmeyer. This will increase the chances of contamination. In addition, this study only tested the physical state of the pH without organoleptic test on the panel. This study would be better if the panelists tested against acceptance. Received power can be a picture of the development of the next product.

There is no significant difference between either pH or LAB insynbiotic fermented milk which being fortified with NaFeEDTA and zn acetate, fe gluconate and zn acetate, as well as synbiotic fermented milk without fortification. From this study, there is a need for organoleptic test to see the quality of the product during the one month period of storage and the need for analysis of pathogenic bacteria other than LAB to compare the presence of good bacteria in synbiotic fermented milk fortified and non-fortified.

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Conflict of Interest

We do not have any conflict of interest to declare.

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