The Activation Method of Lactoperoxidase System to Inhibit Microbial Activity in Fresh Milk

OR Puspitarini*, AN Al-Baarri, AM Legowo, P Bintoro and A Hintono

Faculty of Animal Science and Agriculture; Diponegoro University
Kampus Drh. Soedjono Koesoemowardojo, Tembalang, Semarang 50275 Indonesia
***Corresponding author email: oktaviapuspitarini@yahoo.com; telephone : +6282133503253**

Abstract. Lactoperoxidase system is antimicrobial system in milk. The LPO system has been succesfully applied in tropical regions to prolong the shelf life of milk. However, the LPOS activation is mostly conducted in the first hour of storage. In the case of milk processing manufacture, it takes up to 6 until 7 hours to preserve milk, therefore in this article, LPOS activation is discussed based on different activation time. The initial LPOS activation was conducted at the first and the third storage hour with formula of 0.25 ml of 0.0125 mM SCN^-; 0.25 ml of 0.0125 mM H_2O_2 and 0.5 ml of 35 U/ml LPO in 9 ml of milk and the second activation was conducted with formula of 0.25 ml of 0.0125 mM SCN^-; 0.25 ml of 0.0125 mM H_2O_2 and 0.5 ml of 35 U/ml LPO into 9 ml milk and formula 0.5 ml of 0.05 mM SCN^- ; 0.5 ml of 0.05 mM H_2O_2 and 1 ml of 35 U/ml LPO into 8 ml of milk at 30 °C. The result of research shows that the activation at the third hour with formula of 0.5 ml of 0.05 mM SCN^-; 0.5 ml of 0.05 mM H_2O_2 and 1 ml of 35 U/ml LPO into 8 ml of milk can decrease total milk microbe up to below the standard of total fresh milk microbe that is $5.35x10^3$ CFU/ml and pH level is 6.475. This research indicates that the LPOS activation at the third storage hour with formula of 0.5 ml of 0.05 mM SCN^-; 0.5 ml of 0.05 mM H_2O_2 and 1 ml of 35 U/ml LPO can be used to inhibit the growth of milk microbes at 30 ° C, therefore, milk is safe to be consumed.

Keywords: milk, lactoperoxydase system, lactoperoxidase, total microbes

Abstrak. Sistem laktoperoksidase (LPOS) adalah sistem antimikroba di dalam susu. Sistem LPO ini telah berhasil diterapkan pada daerah tropis untuk memperpanjang masa simpan susu namun aktivasi LPOS banyak dilakukan pada jam pertama penyimpanan. Kasus di industri pengolahan susu, lama pengawetan susu mencapai 6 sampai 7 jam, oleh karena itu artikel membahas aktivasi LPOS berdasarkan waktu aktivasi yang berbeda. Pengaktifan LPOS pertama dilakukan pada jam pertama penyimpanan dan jam ketiga penyimpanan dengan formula 0,0125 mM SCN^- 0,25 ml; 0,0125 mM H_2O_2 0,25 ml dan 35 U/ml LPO 0,5 ml ke dalam 9 ml susu dan pengaktifan kedua dilakukan dengan formula 0,0125 mM SCN^- 0,25 ml; 0,0125 mM H_2O_2 0,25 ml dan 35 U/ml LPO 0,5 ml ke dalam 9 ml susu dan formula 0,05 mM SCN^- 0,5 ml; 0,05 mM H_2O_2 0,5 ml dan 35 U/ml LPO 1 ml ke dalam 8 ml susu pada 30°C. Hasil penelitian menunjukkan bahwa pengaktifan jam ketiga dengan formula 0,05 mM SCN^- 0,5 ml; 0,25 mM H_2O_2 0,5 ml dan 35 U/ml LPO 1 ml ke dalam 8 ml susu mampu menurunkan total mikroba susu hingga di bawah standar total mikroba susu segar yaitu $5,35x10^3$ CFU/ml dan nilai pH yaitu 6,475. Penelitian ini mengindikasi bahwa pengaktifan LPOS pada jam ketiga penyimpanan dengan formula 0,05 mM SCN^- 0,5 ml; 0,25 mM H_2O_2 0,5 ml dan 35 U/ml LPO 1 ml dapat menurunkan total mikroba pada 30°C sehingga susu aman dikonsumsi dan tahan lama.

Kata kunci: susu, sistem laktoperoksidase, laktoperoksidase, total mikroba

Introduction

Milk contains LPOS components; they are lactoperoxidase (LPO), thiocyanate ion (SCN^-), and hidrogen peroxide (H_2O_2). The concentration of LPO enzyme is 30 ppm, SCN^- in milk is 1-15 ppm and H_2O_2 is available in trace amount in milk and it is generated by microbes under aerobic condition, therefore, hydrogen peroxyde should be added in the LPOS activation at a concentration of 100-800 ppm (Seifu et al., 2005; Jooyandeh et al., 2011). We found that LPOS was not stable in milk. LPO activity decreased following the time and temperature of storage. This suggests that the LPO activity decreases due to the increasing

storage time (Puspitarini et al., 2012). The decrease of LPO activity is also found in whey. The activity of LPO lasted 1-2 weeks when whey was stored at 25°C while the activity of LPO in whey that was stored at -20°C, lasted 4 weeks therefore, the decrease of LPO activity was assumed to be due to the increasing temperature of storage (Al-Baarii et al., 2011). Besides, the existences of components in milk such as casein, SCN^- and H_2O_2 also inhibite the LPO activity (Fonteh et al., 2005; Seifu et al., 2005). Therefore, the LPO activity depends in the presence of SCN^- and H_2O_2.

LPO is an oxido-reductase enzyme. This enzyme is capable of catalyzing specific molecules such as hydrogen peroxide of thiocyanate to generate antimicrobial product called hypothiocyanite ($OSCN^-$) (Marshall, 2004; Jooyandeh et al., 2011). $OSCN^-$ is able to break sulfhydryl group of microbe cell that inhibite the growth of bacteria (Seifu et al., 2005, Jooyandeh et al., 2011).

LPO system is an antimicrobial system contained in milk and used to preserve milk especially in condition without cooling (Asaah et al., 2007, Defabachew, 2003, Seifu et al., 2005, Saad, 2008). LPO system is also applied in fish product (Montiel et al., 2012; Jooyandeh et al., 2011) and fermented milk product as in goat fermented milk (Parry-Hanson et al., 2009), cheese (Seifu et al., 2004; Sulieman et al., 2009). LPO system is not only applied in animal food but also in vegetable foods (Touch et al., 2004; Hayashi et al., 2012). The usage of LPOS in food has been done so many times by adding SCN^- and H_2O_2 substrate. Therefore, this research was conducted by adding LPO as enzyme and SCN and H_2O_2 as substrate so that the three components were available to form LPOS and to produce $OSCN^-$.

Most researches studied LPOS activation at the first hour (Asaah et al., 2007; Saad, 2008). Until recently, there has not been found any research about LPOS activation that is based on time based-LPO system activation. Therefore, the aim of this research was to analyse LPOS activation at various activation time in bovine milk. The advantage of this research was to open an alternative method on how LPOS activation in preserving bovine milk using LPOS activation could be applied.

Materials and Research Methods

The fresh milk was from the local dairy farm of Animal and Agriculture Science, Faculty of Animal Science, Diponegoro University. Lactic acid and commercial rennet were derived from Singapore. Potassium thiocyanate (KSCN), hydrogen peroxide (H_2O_2), 2,20-azino-bis(3-ethylbenz-thiazoline-6-sulphonic acid) or ABTS were derived from Kagawa Science (Lot No. 7ROZC-EC) Tokyo Chemical, Industry Co. Ltd. Japan. Sepharose Fast Flow (SP-FF) was derived from Sweden (Lot No. 10029743). The spechtrophotometer (mini UV-1800, Scimadzu, Japan) was used analysis LPO activity.

Preparation of Whey

Two liters of fresh cow's milk was centrifuged at 10.300 x g at 10°C for 30 min. to minimize the fat content. The skim milk was treated with 0.02 % (w/v) rennet and 2.0 ml lactic acid/liter milk at 30°C for 30 min. The precipitated caseins were removed by filtration through a sterilized filter cloth and then through filter paper under vacuum condition. One part of the yield in the form of filtrate was used as whey (Al-Baarri et al., 2010).

Purification of LPO

Whey as much as 2000 ml was flowed into a glass column (3x40 cm) containing 60 g of SP Sepharose Fast Flow (SP- FF) (GE Healthcare Bio-Science AB, Sweeden, Lot. No. 10029743). The whey flow in the column was circulated using a peristaltic pump with a flow rate of 1 ml/min. After the whey was out, SP-FF was washed with 300 ml solution of 0.4 M NaCl in 0.1 M PB to produce lactoperoxidase solution.

The solution of lactoperoxidase was measured in 280 nm using spectrophotometer to determine the concentration of protein. To identify the activity of LPO, spectrophotometer was used at high absorbance using 2,20-azino-bis(3-ethylbenz-thiazoline-6-sulphonic acid) or ABTS in 412nm. The SDS-Page analyze was used to determine the purity of LPO. At the last phase, SP-FF was washed with 1 M of NaCl in 300 ml of distilled water to separate the proteins that was still bound to the resin (Al-Baarri et al., 2010).

Procedure of LPOS Activation

The initial activation was the activation of LPOS at the first and the third storage hour into 4 samples of fresh raw milk that was milk with the activation of LPO, the activation of LPO and SCN^-, the activation of LPO and H_2O_2, and the activation of LPOS then was incubated at the ambient temperature (30°C) for 6 hours. The LPOS components used were 0.25 ml of 0.0125 mM SCN^-, 0.25 ml of 0.0125 mM H_2O_2 and 0.5 ml of 35 U/ml LPO. These components were poured into 9 ml of fresh raw milk.

The second activation was LPOS activation with A formula and B formula into 4 samples of fresh raw milk that was milk with the activation of LPO, the activation of LPO and SCN^-, the activation of LPO and H_2O_2, and the activation of LPOS then it was incubated at the ambient temperature at 30°C for 6 hours. A formula consisted of 0.25 ml of 0.0125 mM SCN^-, 0.25 ml of 0.0125 ml H_2O_2 and 0.5 ml of 35 U/ml LPO. B formula consisted of 0.5 ml of 0.05 mM SCN^- , 0.5 ml of 0.05 mM H_2O_2 and 1 ml of 35 U/ml LPO.

Procedure of Total Microbe Preparation

Petrifilm Aerobic Count Plates (3M Microbiology, St. Paul, Minn., U.S.A) was used to determine the total microbe count of milk. Milk as much as 900 µl was put into a sterile container and mixed with sterile diluents of 100 µl NaCl. This mixture was diluted. The dilution as much as 1000 µl was spread on to the plates on the centre position and pressed for a minute. The sample was incubated for 48 h at the temperature of 37°C.

Results and Discussion

Milk Total Microbe

Storage is one of factors that can cause damage in fresh milk. Total microbe in milk during the six hours can be seen in Figure 1.

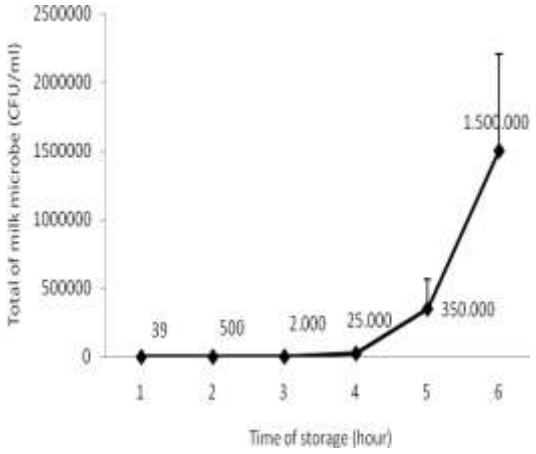

Figure 1. The mean of total milk microbe

The total microbe of milk increased up to 1.500.000 CFU/ml at the sixth hour of storage period at ambient temperature. The increase of milk microbe observed was caused by the absence of resistance system againts microbe. The resistance system meant here was LPO system. The LPO system might be not effective during the six hours and its existence in milk was not active. This happened because three components that form LPO system, namely LPO, SCN^- and H_2O_2, were not stable. The components of H_2O_2 increased during the storage, however, the LPO and the SCN^- lost during 6 hours of storage time. The SCN- decreased as a result that $OSCN^-$ compound was not formed. According to Seifu et al. (2005), the LPO system consists of three components, they are LPO, thiocyanate and hydrogen peroxide. This LPO system will be

active if all the three components are available. This LPO system will generate antimicrobial compound (FAO, 2005; Min et al., 2005; Asaah et al., 2007, Defabachew, 2003, Seifu et al., 2005, Saad, 2008). The possibility $OSCN^-$ compound was not formed during the six hours of storage time indicated that there was no antimicrobial compound in milk that can damage microbe cells. This was in line with Seifu et al. (2005) statement that $OSCN^-$ is able to oxidate SH group of microbe cell. The oxidation of SH group causes the damage of membrane structure of cell sitopalsm, therefore, protein absortion, DNA , and RNA were inihibited. Figure 1 above shows that total milk microbe from the first hour to the sixth hour of storage time always increased. This is in line with Touch et al. (2004) reports that S. Enteritidis microbe in milk that is stored at 30 °C increases as the length of storage time also increases. The total microbe at the first hour of storage time reached 39 CFU/ml and constantly increased up to $1.5x10^6$ CFU/ml at the sixth hour of storage.

Milk Total Microbe by LPOS Activation

Variable of total microbe by the LPOS activation becomes the basis in determining the time of LPOS activation and determining LPOS formula into the fresh raw milk. The discussion of both chapters will be explained further below:

Determining the time of LPOS activation into the fresh raw milk

The determination of the LPOS activation time was based on total microbe in milk at the sixth hour of storage by LPOS activation at the first and the third phase of storage time. The initial total microbe in milk at the sixth hour reached $1.5x10^6$ CFU/ml. The sixth hour was considered as the last stage of analisys due to the local condition or the fact that the transportation from farmers to milk collection spot took approximately six hours. The famers usually milk the cow at 4 a.m. in the morning then delivered and collect the milk at 10.00 a.m. For other cases, farmers that were far away from the milk collection spot needed longer time to reach the collection spot.

LPOS components that were used were 0.25 ml of 0.0125 mM SCN^-; 0.25 ml of 0.0125 mM H_2O_2 and 0.5 ml of 35 U/ml LPO. Total of milk microbe with LPOS activation at the first and third storage hour can be seen in Figure 2. Figure 2 below explains total milk microbe at the sixth hour of storage with LPOS activation at the first hour far above the number of total microbe threshold of 1.000.000 CFU/ml. The third hour was the best time to activate the LPO system into fresh milk because the total microbe that was produced was close to $1x10^6$ CFU/ml.

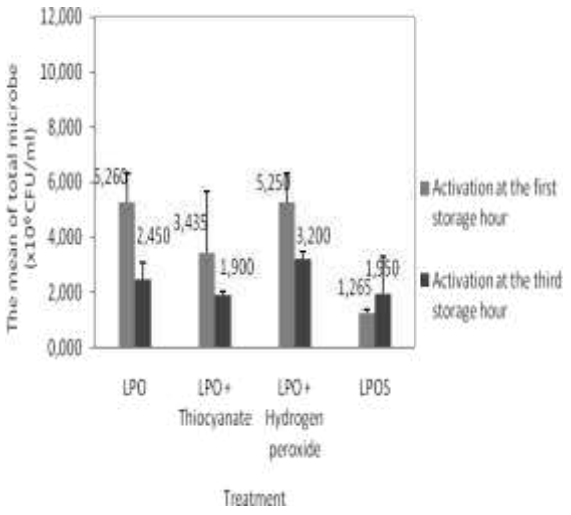

Figure 2. The mean of total milk microbe at the sixth hour of storage with LPOS activation at the first and third storage with 0.25 ml of 0.0125 mM SCN^- and 0.25 ml of 0.0125 mM H_2O_2 and 0.5 ml of 35 U/ml LPO that was stored at ambient temperature 30°C

The determination of the time of LPO addition into milk also considered the concentration of the three components of LPO system in inhibiting the growth of microbe for 6

hours of storage. Based on the existence of the three components of LPO system, the best time to activate the LPO system into fresh milk was the third storage hour. This was because LPO as catalyst of LPO system decreased drastically at the third hour even though both substrates still existed (H_2O_2 increases while SCN^- decreases). When the LPO activity in milk was begining to decrease and disappeared while H_2O_2 and SCN^- compound was still available, then both substrate compounds could not be catalysed by LPO to yield $OSCN^-$ compound as antimicrobial compound. The LPOS activation at the first hour required approximately 5 - 6 hours to resist the milk from microbial activity, while LPOS activation at the third hour required 3 - 6 hours to protect the milk from microbial activity, therefore, the activation of LPOS at the third hour made it possible that LPO and $OSCN^-$ compound could be formed to inhibit microbial activity. This dealt with LPO tenacity in milk that only last for 2 – 3 hours. The LPO cannot last longer, it is not durable. This LPO enzyme catalyses peroxide and thiocyanate to yield products that can kill the growth of microbe (Jooyandeh et al., 2011). Lactoperoxidase has the ability to catalyse certain molecule such as hydrogen peroxide (Marshall, 2004).

The determination of LPOS activation time in milk also considers the time needed by farmers to transport the milk. The cooperative institutions generally accept the milk from farmers approximately at the third hour after milking. The time determination of the LPOS activation also considers the time needed by LPOS components to form $OSCN^-$. $OSCN^-$ concentration as 0.4 mM was reached at 10 times cycles of LPOS reaction. To produce $OSCN^-$ that was stable in 200 ml of whey, ten times of the LPOS reaction time is needed (Al-Baarri et al., 2010). The results showed that the concentration of 0.0125 mM substrate had not been able to inhibit the growth of microbes. Therefore, it was a must to increase the concentration of the substrate to produce 0.4 mM $OSCN^-$ to inhibit microbial growth. The increased concentrations of the substrate must be accompanied by an increase in LPO activity as a catalyst. The balance of the amount and concentration of the substrate with the LPO enzyme is necessary, therefore, there is no much residue accumulates in the body when the product is consumed.

It can be concluded that the time of LPOS activation was determined by considering some factors, such as the concentration of the three components of LPOS in milk, total milk microbe with LPOS activation, the time needed to transport milk from farmers to Village Cooperative, and the time needed to form $OSCN^-$ in milk.

Determination of the concentrations of three compounds in the A and B Formula of LPOS

The results of the research showed that total milk microbe using the LPOS microbe formula was as much as 0.25 ml of 0.0125 mM SCN^-, 0.25 ml of 0.0125 mM H_2O_2, and 0.5 ml of 35 U/ml LPO into 9 ml of milk went over the threshold of total milk microbe that is safe to be consumed. Therefore, the amount formula of LPOS was raised so that the total of milk microbe was below $1x10^6$ CFU/ml, therefore, the milk is safe to be consumed. Based on the SNI 01-6366-2000 the maximum limit of total milk microbe is $1x10^6$ CFU/ml. The total microbe of milk at the sixth hour with A formula of LPOS was as much as 0.25 ml of 0.0125 mM SCN^-, 0.25 ml of 0.0125 mM H_2O_2, 0.5 ml of 35 U/ml LPO and B formula of LPOS 0.5 ml of 0.05 mM SCN^-, 0.5 ml of 0.05 mM H_2O_2 and 1 ml of 35 U/ml LPO that can be seen in Figure 3.

Figure 3 shows that total milk microbe at the sixth hour using 0.25 ml of 0.0125 mM $SCN^-$and 0.25 ml of 0.0125 mM $H_2O_2$and 0.5 ml of 35 U/ml LPO was still above the threshold of the number of total microbe that is 1,949 x 10^6 CFU/ml. The total microbe of milk at the sixth hour with the LPOS activation of 0.5 ml of 0.05

mM SCN^- , 0.5 ml of 0.05 mM $H_2O_2$and 1 ml of 35 U/ml LPO was below the threshold number of total milk microbe of 1.000.000 CFU/ml, as much as $5,3x10^3$ CFU/ml. The decrease of the total number of milk microbe with the LPOS formula was probably caused by LPOS formula 0.5 ml of 0.05 mM SCN^- , 0.5 ml of 0.05 mM $H_2O_2$and 1 ml of 35 U/ml LPO that can form $OSCN^-$ with certain concentration which could inhibit microbial activity in milk, while LPOS with 0.25 ml of 0.0125 mM SCN^- , 0.25 ml of 0.0125 mM H_2O_2 and 0.5 ml of 35 U/ml LPO was not able to produce $OSCN^-$ with certain concentration that could inhibit microbial activity. According to Al-Baarri et al. (2010), $OSCN^-$ concentration of 0,4 mM can yield antimicrobial activity in the six cycles of LPOS reaction. This concentration of 0,4 mM $OSCN^-$ was formed from 1 mM KSCN and 1 mM H_2O_2. It can be said that 1 mM KSCN and 1 mM H_2O_2 yielded 40% $OSCN^-$. The results of this research found that the amount of LPOS 0.25 ml of 0.0125 mM SCN^-, 0.25 ml of 0.0125 mM H_2O_2 and 0.5 ml of 35 U/ml LPO only yielded $OSCN^-$ as much as 0.005 while LPOS with 0.5 ml of 0.05 mM $SCN^-$and 0.5 ml of 0.05 mM H_2O_2 and 1 ml of 35 U/ml LPO produced 0.02 mM $OSCN^-$. Consequently, the best amount formula of LPOS that could be applied into fresh milk was 0.5 ml of 0.05 mM SCN^- , 0.5 ml of 0.05 mM H_2O_2 and 1 ml of 35 U/ml LPO with the activation of all the three components of LPOS.

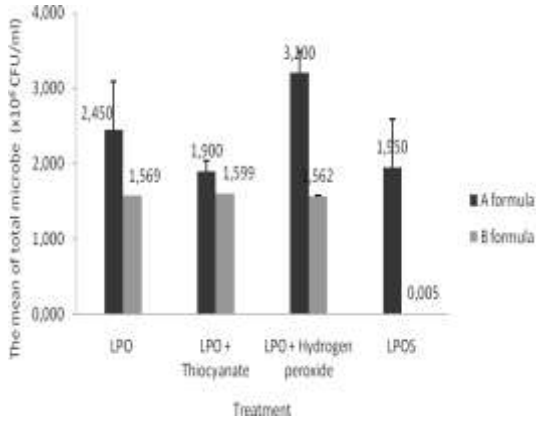

Figure 3. The mean of total milk microbe at the sixth hour with A formula (0.25 ml of 0.0125 mM SCN^- and 0.25 ml of 0.0125 mM H_2O_2 and 0.5 ml of 35 U/ml LPO) and B formula (0.5 ml of 0.05 mM SCN^- , 0.5 ml of 0.05 mM H_2O_2; 1 ml of 35 U/ml LPO) that was stored at ambient temperature of 30 °C

pH Level of Milk

The level of milk pH was observed at the sixth hour. The level of milk pH can be seen in Figure 4. Figure 4 explains that milk with the addition of only LPO or with the addition of LPO and SCN^- decreased the level of milk pH upto below the standard level of fresh milk, while milk that was activated with LPO system resulted in pH level in the range of quality standard of fresh milk, 6.485. The pH level of milk that was stored for 6 hours was 6.075. This meant that the LPOS activation was able to keep pH level of fresh milk. Based on the SNI 01-3141-1998, the requirement of fresh milk quality has 6—7°SH of acidity degree. The level of pH reached in this research was in agreement with that of Sulieman et al. (2009) who stated that the level of pH in fresh milk at the sixth hour reaches 5.94, and the level of pH in milk activated with LPOS at the sixth storage hour reaches the value of 6.05. The level of pH in fresh milk in the recent study was lower compared to pH level of milk activated with LPOS. This was in lines with that of Sulieman et al. (2009), that pH level of control milk compared to milk treated with LPOS that were stored at ambient temperature seemed different. This indicates that preservation of milk with LPOS activation is essential.

With the existence of SCN^-, and H_2O_2 substrate, the addition of LPO system into milk yielded the $OSCN^-$ as the main product, this $OSCN^-$ later will act as antimicrobial compound that can influence the pH level of milk. The activation of LPOS into fresh milk can yield $OSCN^-$ as antimicrobial compound, therefore, the growth of microbe in milk as well as the

forming of acid by microbe can be inhibited. However, when milk was activated with only LPO or activated with LPO and SCN^-, it could not yield $OSCN^-$ compound. Consequently, the growth of microbe increased that resulted in the production of more acids that decreased the pH level.

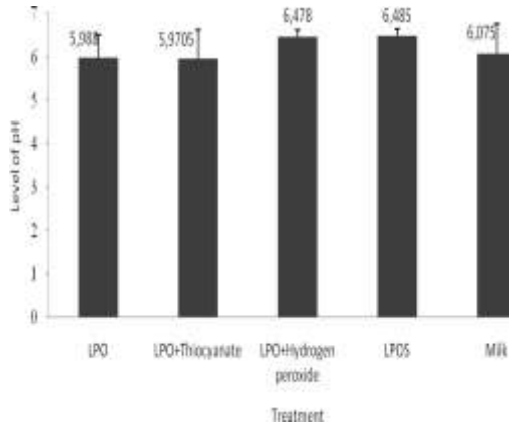

Figure 4.The mean of pH level of milk at the sixth hour with LPOS consisting of 0.5 ml of 0.05 mM SCN^-, 0.5 ml of 0.05 mM H_2O_2 and 1 ml of 35 U/ml LPO stored at ambient temperature 30°C

Conclusions

The activation of LPOS in milk needs three components of LPO as enzyme, SCN^- and H_2O_2 as substrates with the certain concentrations of formula. The uncorrect concentrations of enzyme and substrate yields a not optimum LPOS. From this research, it is concluded that LPOS activation with formulation of 0.5 ml of 0.05 mM SCN^-; 0.5 ml of 0.05 mM H_2O_2 and 1 ml of 35 U/ml LPO into 8 ml of milk at the third storage hour suppresses milk microbial activity up to below the standard of total milk microbe, therefore, milk is safe to be consumed and can last longer.

Acknowledgement

This research was supported by donation from the Ministry of Research and Technology of Republic of Indonesia for “Intensif Riset SINAS”.

References

Al-Baarri AN, M Ogawa and S Hayakawa. 2010. Scale-up studies on immobilization of lactoperoxidase using milk whey for producing antimicrobial agent. J. Indonesian Tropical Animal Agriculture. 35(3):185-191.

Al-Baarri AN, M Ogawa and S Hayakawa. 2011. Application of Lactoperoxidase System Using Bovine Whey and The Effect of Storage Conditions on Lactoperoxidase Activity. J. Dairy Sci. 6(1):72-78.

Asaah NO, F Fonteh, P Kamga, S Mendi and H Imele. 2007. Activation of the lactoperoxidase system as a method of preserving raw milk in areas without cooling facilities. African Journal of Food Agriculture Nutrition and Development. 7: 1-15.

Defabachew ES. 2003. Application of the lactoperoxidase system to improve the quality and safety of goat milk and goat milk cheese. Dissertations. Graduate School, University of Pretoria, Africa.

FAO. 2005. Benefit and Potential Risks of the Lactoperoxidase System of Raw Milk Preservation. Animal Production Service. FAO Animal Poduction and Health Division, Rome.

Fonteh F, A Grandison, and MJ Lewis. 2005. Factors affecting lactoperoxidase activity. Journal of Dairy Technology. 58(4):233-236.

Hayashi M, S Naknukool, S Hayakawa, M Ogawa and AN Al-Baarri. 2012. Enhancement of antimicrobial activity of a lactoperoxidase system by carrot extract and β-carotene. Food Chemistry. 130:541-546.

Jooyandeh H, A Aberoumand and B Nasehi. 2011. Application of lactoperoxidase system in fish and food products: a review. American-Eurasian Journal of Agriculture And Environment Science. 10(1):89-96.

Marshall, K. 2004. Therapeutic applications of whey protein : review. Alternative Medicine Review. 9(2):136-156.

Min, S., L. J. Harris and J. M. Krochta. 2005. Antimicrobial effects of lactoferrin, lysozyme, and the lactoperoxidase system and edible whey protein films incorporating the lactoperoxidase system against *Salmonella enterica* and *Escherichia coli* O157:H7. Journal Food Science. 70(7):332-338.

Montiel R, D Bravo, M de Alba, P Gaya and M Medina. 2012. Combined effect of high pressure treatments and the lactoperoxidase system on

the inactivation of *Listeria monocytogenes* in cold-smoked salmon. Innovative Food Science and Emerging Technologies. 16:26-32.

Parry-Hanson A, P Jooste and E Buys. 2009. Inhibition of Escherichia coli O157:H7 in comercial and traditional fermented goat milk by activated lactoperoxidase. Dairy Science Technologies. 89:613-625.

Puspitarini OR, VY Villa, AN Al-baarri and A Hintono. 2012. Effectiveness of indegenous component lactoperoxidase system in milk. In: Proceeding of Student Conference The Power of Local Knowledge in Increasing Food Business Competitivenes. Semarang, 4th December 2012.Pp:205-212.

Saad AH. 2008. Activation of milk lactoperoxidase system for controlling *pseudomonas* in cow's milk. Journal of Dairy Science. 3(3):131-136.

Seifu E, EM Buys, and EF Donkin. 2004. Quality aspect of gouda cheese made from goat milk preserved by lactoperoxidase system. International Dairy Journal. 14:581-589.

Seifu E, EM Buys and EF Donkin. 2005. Significance of the lactoperoxidase system in the dairy industry and its potential apllications : a review. Trends in Food Science and Technology. 16:137-154.

SNI (Indonesian National Standard). 1998. SNI 01-3141-1998 on Fresh Milk. National Standardization Agency (BSN), Jakarta.

SNI (Indonesian National Standard). 2000. SNI 01-6366-2000 on Microbial Contamination Limit and Limit Maximum Residues in Foodstuffs of Animal Origin. National Standardization Agency (BSN), Jakarta.

Sulieman AME, SE Zubier, and SBE Hardallou. 2009. Activation of lactoperoxidase milk in manufacture of jibna-beida (white cheese). Journal of Science and Technology. 10(1):1-12.

Touch V, S Hayakawa, S Yamada, and S Kaneko. 2004. Effect of a lactoperoxidase-thiocyanate-hydrogen peroxide system on Salmonella enteretidis in animal or vegetable foods. Journal of Food Microbiology. 93:175-183.