

# The activation method of lactoperoxidase system

*by* Ahmad Nimatullah Al-baarri

---

**Submission date:** 15-Nov-2019 02:20AM (UTC+0700)

**Submission ID:** 1213881554

**File name:** The\_activation\_method\_of\_lactoperoxidase\_system.pdf (429.51K)

**Word count:** 4840

**Character count:** 22077

16

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

OR Puspitarini\*, AN Al-Baari, 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

28

**Abstract.** Lactoperoxidase system is an antimicrobial system in milk. The LPO system has been successfully 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<sup>-</sup>; 0.25 ml of 0.0125 mM H<sub>2</sub>O<sub>2</sub> 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<sup>-</sup>; 0.25 ml of 0.0125 mM H<sub>2</sub>O<sub>2</sub> and 0.5 ml of 35 U/ml LPO into 9 ml milk and formula 0.5 ml of 0.05 mM SCN<sup>-</sup>; 0.5 ml of 0.05 mM H<sub>2</sub>O<sub>2</sub> 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<sup>-</sup>; 0.5 ml of 0.05 mM H<sub>2</sub>O<sub>2</sub> 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<sup>3</sup> 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<sup>-</sup>; 0.5 ml of 0.05 mM H<sub>2</sub>O<sub>2</sub> 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, lactoperoxidase 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<sup>-</sup> 0,25 ml; 0,0125 mM H<sub>2</sub>O<sub>2</sub> 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<sup>-</sup> 0,25 ml; 0,0125 mM H<sub>2</sub>O<sub>2</sub> 0,25 ml dan 35 U/ml LPO 0,5 ml ke dalam 9 ml susu dan formula 0,05 mM SCN<sup>-</sup> 0,5 ml; 0,05 mM H<sub>2</sub>O<sub>2</sub> 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<sup>-</sup> 0,5 ml; 0,25 mM H<sub>2</sub>O<sub>2</sub> 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<sup>3</sup> CFU/ml dan nilai pH yaitu 6,475. Penelitian ini mengindikasikan bahwa pengaktifan LPOS pada jam ketiga penyimpanan dengan formula 0,05 mM SCN<sup>-</sup> 0,5 ml; 0,25 mM H<sub>2</sub>O<sub>2</sub> 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<sup>-</sup>), and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). The concentration of LPO enzyme is 30 ppm, SCN<sup>-</sup> in milk is 1-15 ppm and H<sub>2</sub>O<sub>2</sub> is available in trace amount in milk and it is generated by microbes under aerobic condition, therefore,

hydrogen peroxide 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-Baarri et al., 2011). Besides, the existences of components in milk such as casein<sup>22</sup> and H<sub>2</sub>O<sub>2</sub> also inhibit the LPO activity (Fonteh et al., 2005; Seifu et al., 2005). Therefore, the LPO activity depends in the presence of SCN<sup>-</sup> and H<sub>2</sub>O<sub>2</sub>.

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<sup>-</sup>) (Marshall, 2004; Jooyandeh et al., 2011). OSCN<sup>-</sup> is able to break sulfhydryl group of microbe cell that inhibit 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 products<sup>26</sup> in goat fermented milk (Parry-Hanson et al., 2009), cheese (Seifu et al., 2004; Sulieman et al., 2009). LPO system is not only applied<sup>25</sup> 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<sup>-</sup> and H<sub>2</sub>O<sub>2</sub> substrate. Therefore, this research was conducted by adding LPO as enzyme and SCN<sup>-</sup> and H<sub>2</sub>O<sub>2</sub> as substrate so that the three components were available to form LPOS and to produce OSCN<sup>-</sup>.

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<sup>20</sup> (KSCN), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), 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 spectrophotometer (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<sup>13</sup> 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  $\text{SCN}^-$ , the activation of LPO and  $\text{H}_2\text{O}_2$ , and the activation of LPOS then was incubated at the ambient temperature ( $30^\circ\text{C}$ ) for 6 hours. The LPOS component used were 0.25 ml of 0.0125 mM  $\text{SCN}^-$ , 0.25 ml of 0.0125 mM  $\text{H}_2\text{O}_2$  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  $\text{SCN}^-$ , the activation of LPO and  $\text{H}_2\text{O}_2$ , and the activation of LPOS then it was incubated at the ambient temperature at  $30^\circ\text{C}$  for 6 hours. A formula consisted of 0.25 ml of 0.0125 mM  $\text{SCN}^-$ , 0.25 ml of 0.0125 ml  $\text{H}_2\text{O}_2$  and 0.5 ml of 35 U/ml LPO. B formula consisted of 0.5 ml of 0.05 mM  $\text{SCN}^-$ , 0.5 ml of 0.05 mM  $\text{H}_2\text{O}_2$  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  $\mu\text{l}$  was put into a sterile container and mixed with sterile diluents of 100  $\mu\text{l}$  NaCl. This mixture was diluted. The dilution

as much as 1000  $\mu\text{l}$  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  $37^\circ\text{C}$ .

## 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.

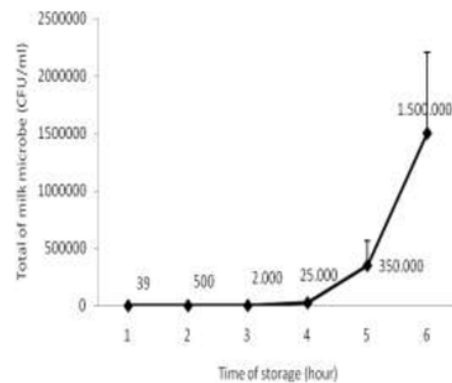


Figure 1. The mean of total milk microbe

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 againsts 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,  $\text{SCN}^-$  and  $\text{H}_2\text{O}_2$ , were not stable. The components of  $\text{H}_2\text{O}_2$  increased during the storage, however, the LPO and the  $\text{SCN}^-$  lost during 6 hours of storage time. The  $\text{SCN}^-$  decreased as a result that  $\text{OSCN}^-$  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<sup>-</sup> 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<sup>-</sup> 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 inhibited. 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<sup>6</sup> 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<sup>6</sup> 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<sup>-</sup>; 0.25 ml of 0.0125 mM H<sub>2</sub>O<sub>2</sub> 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<sup>6</sup> CFU/ml.

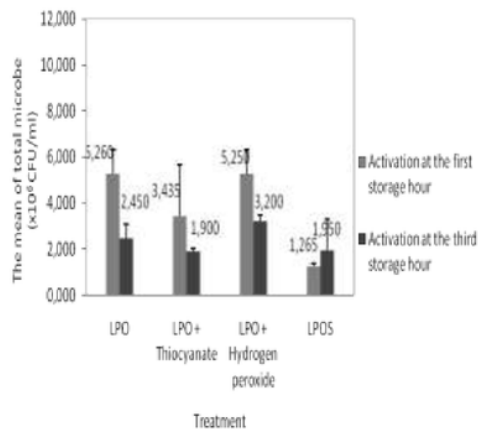


Figure 2. The mean of total milk microbe at the sixth hour of storage with LPOS activation at the first and third storage with 0.25 ml of 0.0125 mM SCN<sup>-</sup> and 0.25 ml of 0.0125 mM H<sub>2</sub>O<sub>2</sub> and 0.5 ml of 35 U/ml LPO that was stored at ambient temperature 30°C

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 beginning 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<sup>-</sup> 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<sup>-</sup> 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<sup>-</sup>. OSCN<sup>-</sup> concentration as 0.4 mM was reached at 10 times cycles of LPOS reaction. To produce OSCN<sup>-</sup> that was stable in 200 ml of whey, ten times <sup>g</sup> the LPOS reaction time is needed (Al-Baari 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<sup>-</sup> 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<sup>-</sup> 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  $1 \times 10^6$  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  $1 \times 10^6$  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  $1,949 \times 10^6$  CFU/ml. The total microbe of milk at the sixth hour with the LPOS activation of 0.5 ml of 0.05

mM SCN<sup>-</sup>, 0.5 ml of 0.05 mM H<sub>2</sub>O<sub>2</sub> 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<sup>3</sup> 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<sup>-</sup>, 0.5 ml of 0.05 mM H<sub>2</sub>O<sub>2</sub> and 1 ml of 35 U/ml LPO that can form OSCN<sup>-</sup> with certain concentration which could inhibit microbial activity in milk, while LPOS with 0.25 ml of 0.0125 mM SCN<sup>-</sup>, 0.25 ml of 0.0125 mM H<sub>2</sub>O<sub>2</sub> and 0.5 ml of 35 U/ml LPO was not able to produce OSCN<sup>-</sup> with certain concentration that could inhibit microbial activity. According to Al-Baarri et al. (2010), OSCN<sup>-</sup> concentration of 0,4 mM can yield antimicrobial activity in the six cycles of LPOS reaction. This concentration of 0,4 mM OSCN<sup>-</sup> was formed from 1 mM KSCN and 1 mM H<sub>2</sub>O<sub>2</sub>. It can be said that 1 mM KSCN and 1 mM H<sub>2</sub>O<sub>2</sub> yielded 40% OSCN<sup>-</sup>. The results of this research found that the amount of LPOS 0.25 ml of 0.0125 mM SCN<sup>-</sup>, 0.25 ml of 0.0125 mM H<sub>2</sub>O<sub>2</sub> and 0.5 ml of 35 U/ml LPO only yielded OSCN<sup>-</sup> as much as 0.005 while LPOS with 0.5 ml of 0.05 mM SCN<sup>-</sup> and 0.5 ml of 0.05 mM H<sub>2</sub>O<sub>2</sub> and 1 ml of 35 U/ml LPO produced 0.02 mM OSCN<sup>-</sup>. Consequently, the best amount formula of LPOS that could be applied into fresh milk was 0.5 ml of 0.05 mM SCN<sup>-</sup>, 0.5 ml of 0.05 mM H<sub>2</sub>O<sub>2</sub> and 1 ml of 35 U/ml LPO with the activation of all the three components of LPOS.

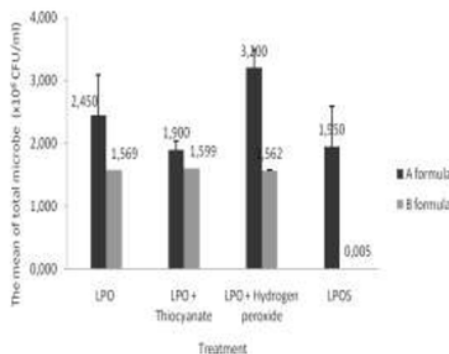


Figure 3. The mean of total milk microbe at the sixth hour with A formula (0.25 ml of 0.0125 mM SCN<sup>-</sup> and 0.25 ml of 0.0125 mM H<sub>2</sub>O<sub>2</sub> and 0.5 ml of 35 U/ml LPO) and B formula (0.5 ml of 0.05 mM SCN<sup>-</sup>, 0.5 ml of 0.05 mM H<sub>2</sub>O<sub>2</sub>; 1 ml of 35 U/ml 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<sup>-</sup> 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<sup>-</sup>, and H<sub>2</sub>O<sub>2</sub> substrate, the addition of LPO system into milk yielded the OSCN<sup>-</sup> as the main product, this OSCN<sup>-</sup> later will act as antimicrobial compound that can influence the pH level of milk. The activation of LPOS into fresh milk can yield OSCN<sup>-</sup> 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<sup>-</sup>, it could not yield OSCN<sup>-</sup> compound. Consequently, the growth of microbe increased that resulted in the production of more acids that decreased the pH level.

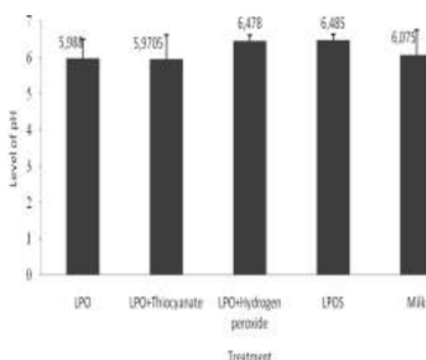


Figure 4. The mean of pH level of milk at the sixth hour with LPOS consisting of 0.5 ml of 0.05 mM SCN<sup>-</sup>, 0.5 ml of 0.05 mM H<sub>2</sub>O<sub>2</sub> and 1 ml of 35 U/ml LPO stored at ambient temperature 30°C

## Conclusions

The activation of LPOS in milk needs three components of LPO as enzyme, SCN<sup>-</sup> and H<sub>2</sub>O<sub>2</sub> 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<sup>-</sup>; 0.5 ml of 0.05 mM H<sub>2</sub>O<sub>2</sub> 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 Production 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  $\beta$ -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):335-338.
- 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.
- 8 Parry-Hanson A, P Jooste and E Buys. 2009. Inhibition of *Escherichia coli* O157:H7 in commercial 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 indigenous component lactoperoxidase system in milk. In: Proceeding of Student Conference The Power of Local Knowledge in Increasing Food Business Competitiveness. Semarang, 4<sup>th</sup> December 2012. P. 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 applications : a review. *Trends in Food Science and Technology*. 16:137-154.
- 14 SNI (Indonesian National Standard). 1998. SNI 01-3141-1998 on Fresh Milk. National Standardization Agency (BSN), Jakarta.
- 7 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.
- 2 Touch V, S Hayakawa, S Yamada, and S Kaneko. 2004. Effect of a lactoperoxidase-thiocyanate-hydrogen peroxide system on *Salmonella enteritidis* in animal or vegetable foods. *Journal of Food Microbiology*. 93:175-183.

# The activation method of lactoperoxidase system

---

## ORIGINALITY REPORT

---

16%

SIMILARITY INDEX

%

INTERNET SOURCES

16%

PUBLICATIONS

%

STUDENT PAPERS

---

## PRIMARY SOURCES

---

- 1** Mecitoglu, C.. "Partial purification and preparation of bovine lactoperoxidase and characterization of kinetic properties of its immobilized form incorporated into cross-linked alginate films", Food Chemistry, 2007 3%

Publication
- 2** Al-Baarri, Ahmad Nimatullah, Makoto Hayashi, Masahiro Ogawa, and Shigeru Hayakawa. "Effects of Mono- and Disaccharides on the Antimicrobial Activity of Bovine Lactoperoxidase System", Journal of Food Protection, 2011. 2%

Publication
- 3** Al-Baarri, Ahmad Ni'matullah, Anang Mohamad Legowo, Shigeru Hayakawa, and Masahiro Ogawa. "Enhancement Antimicrobial Activity of Hyphothiocyanite Using Carrot Against Staphylococcus Aureus and Escherichia Coli", Procedia Food Science, 2015. 2%

Publication
- 4** Ali Ehsani, Mohammad Hashemi, Asma Afshari, Majid Aminzare, Mojtaba Raeisi, Tayebeh 1%

Zeinali. "Effect of different types of active biodegradable films containing lactoperoxidase system or sage essential oil on the shelf life of fish burger during refrigerated storage", LWT, 2020

Publication

---

5

Raquel Montiel, Izaskun Martín-Cabrejas, Margarita Medina. "Reuterin, lactoperoxidase, lactoferrin and high hydrostatic pressure on the inactivation of food-borne pathogens in cooked ham", Food Control, 2015

Publication

---

6

Ndambi, OA, PB Kanga, H ImelÃ©, SD Mendi, and FA Fonteh. "Effects of milk preservation using the lactoperoxidase system on processed yoghurt and cheese quality", African Journal of Food Agriculture Nutrition and Development, 2008.

Publication

---

7

Ahmad Ni'matullah Al-Baarri, Anang Mohamad Legowo, Septinika Kurnia Arum, Shigeru Hayakawa. "Extending Shelf Life of Indonesian Soft Milk Cheese (Dangke) by Lactoperoxidase System and Lysozyme", International Journal of Food Science, 2018

Publication

---

8

C.L.S. Macuamule, I.J. Wiid, P.D. van Helden,

<1%

M. Tanner, R.C. Witthuhn. "Effect of milk fermentation by kefir grains and selected single strains of lactic acid bacteria on the survival of Mycobacterium bovis BCG", International Journal of Food Microbiology, 2016

Publication

---

9

C. Muro Urista, R. Álvarez Fernández, F. Riera Rodríguez, A. Arana Cuenca, A. Téllez Jurado. "Review: Production and functionality of active peptides from milk", Food Science and Technology International, 2011

Publication

---

<1%

10

Ahmad Ni'matullah Al-Baarri, Novia Tri Damayanti, Anang Mohamad Legowo, Ismail Hakkı Tekiner, Shigeru Hayakawa. "Enhanced Antibacterial Activity of Lactoperoxidase–Thiocyanate–Hydrogen Peroxide System in Reduced-Lactose Milk Whey", International Journal of Food Science, 2019

Publication

---

<1%

11

Amir Golmohamadi, Matthew J. Morra, Inna Popova, Caleb I. Nindo. "Optimizing the use of Sinapis alba seed meal extracts as a source of thiocyanate (SCN<sup>-</sup>) for the lactoperoxidase system", LWT - Food Science and Technology, 2016

Publication

---

<1%

12

A N Al-Baarri, A M Legowo, Widayat, S B M Abduh, M Hadipernata, Wisnubroto, D K Ardianti, M N Susanto, M Yusuf, E K Demasta. "Determination Hypoiodous Acid (HIO) By Peroxidase System Using Peroxidase Enzyme", IOP Conference Series: Earth and Environmental Science, 2018

Publication

&lt;1%

13

Visalsok Touch, Shigeru Hayakawa, Satoshi Yamada, Souichi Kaneko. "Effects of a lactoperoxidase–thiocyanate–hydrogen peroxide system on Salmonella enteritidis in animal or vegetable foods", International Journal of Food Microbiology, 2004

Publication

&lt;1%

14

Nurliyani, , Yuni Suranindyah, and Priyanti Pretiwi. "Quality and Emulsion Stability of Milk from Ettawah Crossed Bred Goat During Frozen Storage", Procedia Food Science, 2015.

Publication

&lt;1%

15

Seifu, E.. "Potential of lactoperoxidase to diagnose subclinical mastitis in goats", Small Ruminant Research, 200705

Publication

&lt;1%

16

Bernard Faye, Mohammed Bengoumi. "Camel Clinical Biochemistry and Hematology", Springer Nature America, Inc, 2018

&lt;1%

17

Hannu J. Korhonen. "Health-Promoting Proteins and Peptides in Colostrum and Whey", Wiley, 2010

Publication

---

<1%

18

Jennifer A. Runquist, Paul A. Loach. "Catalysis of electron transfer across phospholipid bilayers by iron-porphyrin complexes", Biochimica et Biophysica Acta (BBA) - Bioenergetics, 1981

Publication

---

<1%

19

H.-H. Jin. "Combined Effect of Aqueous Chlorine Dioxide and Modified Atmosphere Packaging on Inhibiting Salmonella Typhimurium and Listeria monocytogenes in Mungbean Sprouts", Journal of Food Science, 10/26/2007

Publication

---

<1%

20

Makoto Hayashi, Supaporn Naknukool, Shigeru Hayakawa, Masahiro Ogawa, Al-Baarri Ahmad Ni'matulah. "Enhancement of antimicrobial activity of a lactoperoxidase system by carrot extract and  $\beta$ -carotene", Food Chemistry, 2012

Publication

---

<1%

21

E.H. Bosch. "The lactoperoxidase system: the influence of iodide and the chemical and antimicrobial stability over the period of about 18 months", Journal of Applied Microbiology,

<1%

8/2000

Publication

---

22

TAHIR YAQUB. "The influence of time elapsed after milking and lactation stage on the Lactoperoxidase System of Milk in Sahiwal Cattle and Nili-Ravi Buffaloes", International Journal of Dairy Technology, 03/2012

Publication

---

23

Amrita Poonia. "chapter 14 Antimicrobial Edible Films and Coatings for Fruits and Vegetables", IGI Global, 2017

Publication

---

24

Korhonen, Hannu J., and Pertti Marnila. "Milk Bioactive Proteins and Peptides", Milk and Dairy Products in Human Nutrition Production Composition and Health, 2013.

Publication

---

25

Maria Rosaria Corbo. "Prolonging microbial shelf life of foods through the use of natural compounds and non-thermal approaches - a review", International Journal of Food Science & Technology, 02/2009

Publication

---

26

Carmen A. Campos, Lía N. Gerschenson, Silvia K. Flores. "Development of Edible Films and Coatings with Antimicrobial Activity", Food and Bioprocess Technology, 2010

<1%

<1%

<1%

<1%

<1%

**27** Mohamed, CissÃ©, Kouakou Amenan  
Clementine, Montet Didier, Loiseau GÃ©rard,  
and Ducamp-Collin Marie NoÃ«lle. <math><1\%</math>  
"Antimicrobial and physical properties of edible  
chitosan films enhanced by lactoperoxidase  
system", Food Hydrocolloids, 2013.  
Publication

---

**28** Seifu, E.. "Significance of the lactoperoxidase  
system in the dairy industry and its potential  
applications: a review", Trends in Food Science  
& Technology, 200504 <math><1\%</math>  
Publication

---

**29** Moatsou, Golfo. "Sanitary Procedures, Heat  
Treatments and Packaging", Milk and Dairy  
Products in Human Nutrition Production  
Composition and Health, 2013. <math><1\%</math>  
Publication

---

Exclude quotes Off

Exclude matches Off

Exclude bibliography Off