Al-Baarri

by Enhancement Antimicrobial Activity Of Hyphothiocya Ahmad Ni'matullah

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Enhancement Antimicrobial Activity of Hyphothiocyanite using Carrot Against *Staphylococcus aureus* and *Escherichia coli*

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Abstract

Hypothiocyanite has been known as antimicrobial agent that was generated from lactoperoxidase system (LPOS) but its antimicrobial activity was low against pathogenic bacteria in milk. This research has been done to enhance the antimicrobial activity of hypothiocyanite against pathogenic bacteria commonly found in milk: *Staphylococcus aureus* and *Escherichia coli* by addition of carrot extract. The result showed that carrot extract was able to enhance the antimicrobial activity of hypothiocyanite strongly against *E. coli,* however less enhancement has been found in the antibacterial activity of hypothiocyanite against *S. aureus.*

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Keywords: Lactoperoxidase, antimicrobial activity, carrot extract, *S. aureus*, *E. coli.*

Introduction

Hyphothiocyanite has been well studied as food preservatives that was exhibited from the system namely lactoperoxidase system (LPOS) [1-3]. This antimicrobial agent is able for preserving food without undesirable side effects [4-6]. Therefore the application is now initialized for industrial use. The LPOS consists of LPO, H_2O_2, and SCN^- which is able exhibit hypothiocyanite [7]. The hypothiocyanite has been documented as preservatives in various dairy products such as milk, cheese, and yogurt, and non-dairy products such as mango and vegetable juice [2, 6, 8-14].

Although it was successfully done for preservatives, previous researchs were unable to inhibit the proliferation of pathogenic bacteria in milk (fresh and skim milk). This may be explained by the less power of hyphothiocyanite and the presence of sugar which has been proved as LPO inhibitor [15]. Then the research was continued to the enhancement of the action of LPOS product. Extract carrot has been showed to exhibit the enhancement of antimicrobial activity of LPOS [16]

It was documented that the addition of 20-fold diluted carrot extract boosted LPOS antimicrobial activity from 1.4 to 3.8 log units agaist *S. enteritidies*. The dilution ratio lower than 10-

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fold dilution remarkably increased antimicrobial activity to 6.0 log units. Since the various pathogenic bacteria may be found in milk, the exploration against other pathogenic bacteria such as *S. aureus* and *E. coli* should be performed. This research has been done for enhancing antimicrobial activity of LPOS by the involvement of carrot extract against *S. aureus* and *E. coli*. The improvement may contribute to suppress the cost of LPO.

Materials and Methods

Purification of LPO

Skimmed milk was obtained from two liters of fresh cow's milk that was defatted by centrifugation at 10,300 × g at 10˚C for 30 min. Then, the skim milk was clotted with 0.02% (w/v) rennet and 2 ml lactic acid per liter milk at 30˚C for 30 min. Whey was obtained by removing curd and filtration through filter paper under vacuum conditions. The rest of LPO purification has been conducted as previous research [15].

Enzymatic activity assay

LPO enzymatic activity was determined using ABTS as a method which has been performed by Al-Baarri et al. [7].

Determination of [$OSCN^-$]

$OSCN^-$ concentration was determined according to the method of Al-Baarri et al. [7] with minor modifications. The principle of the method was based on the oxidation of Nbs to Nbs2. Nbs stock solution was prepared by adding 2.0 µl of mercaptoethanol to 10.0 ml of Nbs solution diluted to 0.5 mM with 0.1 M PB (pH 7.2) containing 5.0 mM EDTA (PBE). The Nbs stock solution was prepared fresh daily and kept on ice. Before OSCN− determination, H_2O_2 present in a sample was removed by adding 20 µl of 1.0 mg/ml catalase solution to 1.0 ml sample. Four milliliter of PBE was added to 0.1 ml of the H_2O_2-free sample solution, followed by the addition of 0.5 ml of Nbs stock solution. Immediately, the absorbance of the mixture was measured at 412 nm. The concentration of remaining Nbs was calculated from the absorbance reading, with assumption of a molar absorption coefficient of 13,600 M-1 cm-1 for Nbs.

Preparation of carrot extract

Fresh carrots were peeled and homogenized with 5-fold weight of sterile 0.1 M PB (pH 7.0), containing 0.15 M NaCl. The suspension was centrifuged at 8000g, at 4 °C for 15 min. The resultant supernatant (carrot extract), was 2–20 times be diluted with the same buffer. The carrot extract stock solution, and its diluted solutions, was used for further experiments. All the processes of preparation has been done under aseptic conditions [16].

Production of hypothiocyanite

Hypothiocyanite was generated using the reaction of LPOS components: 10 U of LPO, 0.3 mM KSCN, and 0.3 mM H_2O_2 under aseptic condition [17].

Result and Discussion

It is has been documented that carrot extract was able to stabilize the hypothiocyanite [16] therefore this research tried to calculate the hyphothiocyanite against time of storage in the presence of carrot extract. The result of hypothiocyanite concentration against time of storage is presented in Figure 1.

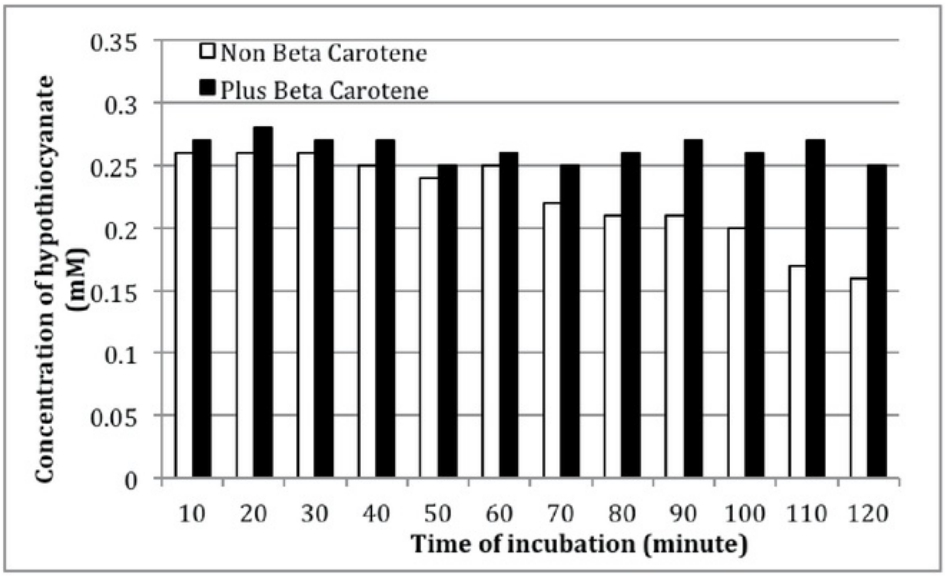

Figure 1. Production of hypothiocyanite from the LPOS solution: LPO (10 U), KSCN (0.3 mM) and H_2O_2 (0.3 mM) with and without extract carrot addition against time of incubation at 37˚C. Values are means of three sets of experiments.

Figure 1 shows the hypothiocyanite production from LPOS against time of incubation for 120 minute at 37˚C with and without the presence of carrot extract. As can be seen, hypothiocyanite concentration ranged from 1.16 to 0.27 mM was able to be generated using LPOS component: 10 U of LPO, 0.3 mM KSCN, and 0.3 mM H_2O_2. The hypothiocyanite concentration gradually increased along the time of incubation, ranging from 1 to 9 minutes (Data were not shown).

As can be seen on Figure 1, carrot extract hindered the reduction of hypothiocyanite concentration. During the 120 minute of incubation, there was a very less amount of losing the hypothiocyanite concentration, however, the remarkable loss of hypothiocyanite was found in the solution without carrot extract (from 0.26 to 0.16 mM). It is proved that carrot extract has a such function to retained the hypothiocyanite in the solution. This might be explained by the enhancement of hypothiocyanite concentration by the production of oxidized beta carotene in the LPOS solution [16]. The oxidized beta carotene would likely react with the remaining SCN, resulting in the generation of hyphothiocyanite.

Figure 2 and 3 shows the antibacterial activity of LPOS against pathogenic bacteria *E. coli* and *S. aureus*, respectively, in the presence and absence of carrot extract. It can be seen that carrot extract exhibited enhancement antibacterial activity of hypothiocyanite in both pathogenic bacteria. However, The remarkable enhancement of antibacterial activity of LPO by carrot extract could be found in *E. coli*. Those remarkable enhancement could be found clearly in the LPOS solution containing the initial count of 5 Log CFU/ml. Carrot extract decreased the population of *E. coli* from 3.8 log CFU/ml to 2.45 log CFU/ml indicating the reduction as much as 0.75 log CFU/ml. However, the enhancement of antibacterial activity of hypothiocyanite by carrot extract was hindered against *S. aureus*.

The enhancement of antibacterial activity by carrot extract has been studied previously [16]. The concurrent addition of beta carotene through carrot extract addition to the LPOS is presumed to be oxidized by the actions of hypothiocyanite which was developed by LPO Compound-I, thiocyanate and H_2O_2. The oxidative reaction from beta carotene to oxidized-beta-carotene brings about the accumulation of thiocyanate, which may be utilized by the remaining LPO Compound-I

resulting in the increase of production hypothiocyanite. This may explain the enhancement antibacterial activity by carrot extract.

Time of addition of carrot extract seems key role for the enhancement since the utilization of LPO substrate (thiocyanate and H_2O_2) to produce hypothiocyanite is time dependent [6, 18]. This might result in the differences on the antibacterial activity of hypothiocyanite. The short time of incubation resulted in the no antibacterial activity of hypothiocyanite (Data were not shown).

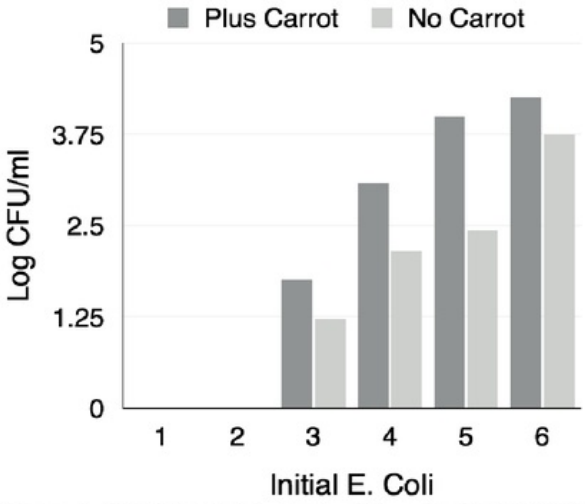

Figure 2. Final bacterial count of *E. coli* after LPOS treatments. Various initial population of *E. coli* (Log CFU/ml) was inoculated to LPOS solution with and the absence of carrot extract in the LPOS solution. The bacteria-contained-solution was incubated for 4 h at 37˚C. Values are means of three sets of experiments.

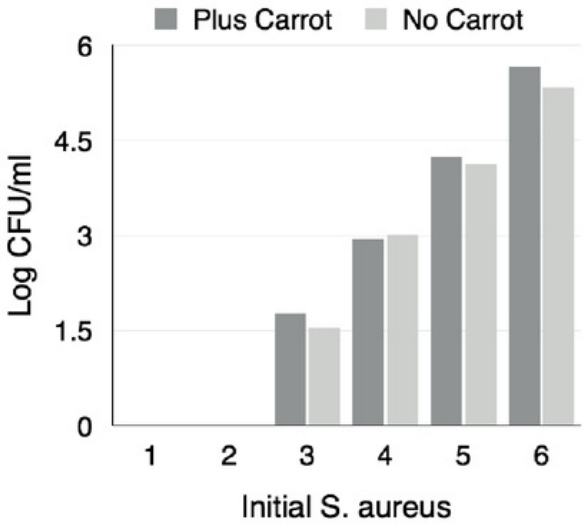

Figure 3. Final bacterial count of *S. aureus* after LPOS treatments. Various initial population of *S. aureus* (Log CFU/ml) was inoculated to LPOS solution with and the absence of carrot extract in the LPOS solution. The bacteria-contained-solution was incubated for 4 h at 37˚C. Values are means of three sets of experiments.

Conclusion

Carrot extract stabilized the presence of hypothiocyanite in the LPOS solution during 120 minute of storage. Furthermore, carrot extract enhanced the antibacterial activity of hypothiocyanite against *E. coli* and *S. aureus.*

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