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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.