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EFFECT OF LACTOPEROXIDASE SYSTEM ON ESCHERICHIA COLI 0111:B4 IN UHT MILK

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Introduction

There are so many difficulties in cooling system and storage and Transportation of raw milk in many developing countries including Iran because of shortage of energy and equipments. As a result the hygienic quality of raw milk decrease before delivered to the factory and sometime it leads to spoilage of raw milk(1). LP system is a natural antimicrobial system in milk which increases the shelf life of raw milk and dairy products (16). E coli O111: B4 has been considered as the target microorganism in this study to evaluate the proper concentration of LP system according to its antimicrobial effect. E.coli O111 belongs to enteropathogenic bacteria and has been isolated from milk and dairy products (12, 13).

Materials and Methods

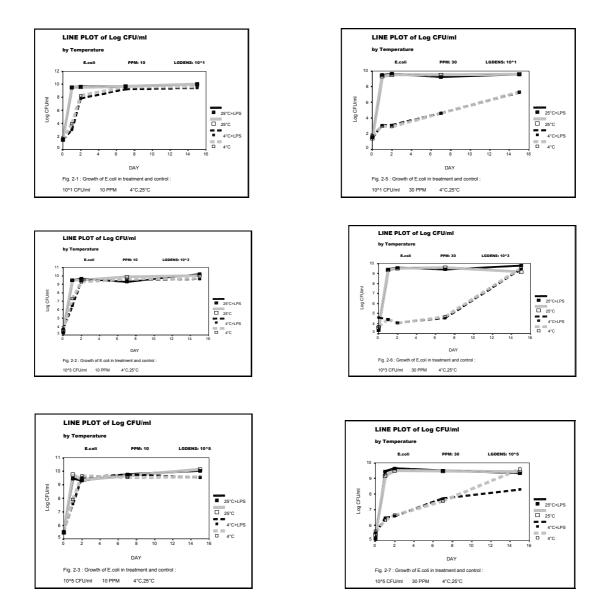
E coli O111:B4 which was obtained from Razi Research Institute (RITCC 1176). Horse Radish loctoperokidase (E C.1.11.1.17) Sigma/Potassium thiocyanate (5124 Merck)/ Hydrogen peroxide 30% (822287 Merck)/ violet Red Bile Dextrose Agar (Merck)/ Brain Heart Infusion Broth (Merck) were used. Different concentration of E coli $(10^1 - 10^3 - 10^5 - 10^7 \text{CFU/ml})$ were inoculated into the UHT milk which contained actives LPS to study the effect of the system on the microorganism. Two concentrations of LP (10 and 30 ppm) which are the naturally accrued of this enzyme in raw milk were prepared and added to the UHT milk together with 0.25 mM of thiocyanate and 0.25 mM of hydrogen peroxide,. All the samples and control were stored at 4 °C and 25 °C and tested at 0,1,2,7 and 14 days intervals. For each sample and control two repetitions were considered.

Results

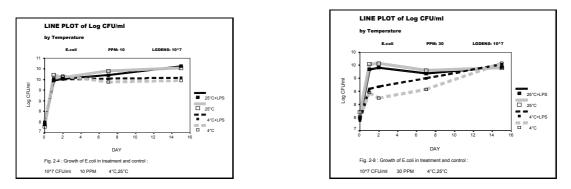
The results have been shown in picture2-1. Groupe 1 is in 25 C and group 2 is in 4 C with 10 ppm of enzyme. The growth has been shown together in the same time in inoculation of 10 1 CFU/ml of bacteria (10 1 CFU/ml + LPS) and It shows that LP system has no effect on E coli. It was very mild growth in 4 C in the samples which shows that LP system is not effective in this temperature. Picture 2-2 shows growth in group 1 and group 2 with 10 3 CFU/ml of E coli

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and 10 ppm of enzyme in 4 C and 25 °C which indicates the LP is not effective. Picture 2-3 and 2-4 show groups 1 and 2 with 10^5 and 10^7 CFU/ml which have shown the same increase in control and sample. The LP system was not effective on E coli in both temperatures. Picture 2-5 and 2-6 show groups 3 (in25^oC with 30 ppm of enzyme) and group 4(4^oC with 30 ppm of enzyme)with same results in 10^1 and 10^3 inoculation and picture 2-7 and 2-8 show groups 3 and 4 with 10^5 and 10^7 CFU/ml which indicate that LP system has no considerable antimicrobial effect on E coli.



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Discussion

The recent studies show that milk and dairy products have certain role in transmission of food borne pathogens such as enteropathogenic E coli in human(2). There are different ideas about effect of LP system on E coli. In one study E coli (NCTC 9703) was not able to survive in presence of hypothiocyonate (11). In another research add of LP system components and activate of LP system in sterilized milk decrease E coli O157:H7 and it shows 2 log decreases in 15 C and 17 C (4). In 1980 Bjorek reported that the LP system has reversible inhibitory effect in many of the gram positive bacteria and irreversible inhibitory effect or bactericidal effect on gram negative bacteria like E coli (3). In another study activation of LP system leads to inhibition of staphylococcus and E coli growth and decrease 2 log of E coli (10). In 1992 Farrag et al., reported that LP system has inhibitory and bacteriocidal effects on E coli O157:H7. The bacteria were totally suppressed when its population was 10⁴ CFU/ml and its growth was inhibited when its number was 10^8 CFU/ml. The LP system is more active in 30 $^{\circ}$ C than 4 °C which is optimal condition for growth and division of cells. The bacteriocidal effect of LP system increase by decrease of pH and it shows why LP system is faster in 30 C (8). The bacteriocidal effect of LP system has been reported on different serotypes of E coli (3, 8). LP system has bactericide effect on E coli CRA646 and S.typhimurium (6). It has been shown that LP system could delay the logarithmic phase of E coli in infant milk powder (7). In one study E coli did not grow in raw milk in 4 C and the effect of LP system was not remarkable (14). In 1997, Denis et al., have studied the LP system effect on different microorganism and results showed that the LP system has no effect on E coli (5). The LP system has inhibitory effect on growth of gram positive bacteria. In this system has bactericidal effect gram negative bacteria like pseudomonas and E coli. The effect of LP system on gram positive bacteria is bacteriostatic but it has bactericidal effect on pseudomonas and E coli (9). In present study it has been shown that LP system has no remarkable effect on this serotype and it is suggested that antimicrobial effect of LP system is related to serotype and genus of microorganism.

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