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β-lactoglobulin and *α*-lactalbumin Hydrolysates as Sources of Antibacterial Peptides

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ABSTRACT

The presence of antibacterial activity in bovine *β*-lactoglobulin and in *α*-lactalbumin hydrolysates was investigated. The Plasmin-Digest of *β*-lactoglobulin (PDβ) and of *α*-lactalbumin (PDα) were fractionated, using reversed phase high performance liquid chromatography. The antibacterial activity of *β*-lactoglobulin, *α*-lactalbumin, nisin, plasmin, PDβ and PDα were *in vitro* tested against pathogenic (*Escherichia coli* and *Staphylococcus aureus*) and probiotic (*Lactobacillus casei* and *Lactobacillus acidophilus*) bacteria. Although *α*-lactalbumin, *β*-lactoglobulin and plasmin exhibited no antibacterial activity, PDβ, PDα and nisin revealed antibacterial activity against the bacteria tested. The Minimum Inhibitory Concentration (MIC) of these compounds was determined for the bacteria cultures. Similar to nisin, the MIC of PDβ and of PDα against Gram-positive bacteria was recorded as considerably lower than the MICs against Gram-negative bacteria. The study also evaluated the effect of PDβ, PDα and nisin on the growth curves and on the plate count confirmations of the target bacteria. The results revealed that nisin, PDβ and PDα have inhibitory effects on the lag phase, maximum OD620 and on plate count confirmation of the bacteria tested. The maximum inhibitory effect of these compounds was created during the log phase. Their inhibitory effects depended upon their concentrations, higher concentration causing stronger antibacterial activity. The PDβ and PDα proved more active against Gram-negative bacteria than did nisin, but nisin revealed substantial inhibitory activity against Gram-positive bacteria.

Keywords: *α*-lactalbumin, Antibacterial, *β*-lactoglobulin, Bovine, Plasmin.

INTRODUCTION

The antibacterial properties of milk have been known for long time. As for the neonate, milk is known to provide not only excellent nutrition but also protection against infections (Lopez-Exposito and Recio, 2006). The antibacterial activity of milk is mainly attributed to the immunoglobulins, but the non-immune proteins, lactoferrin, lactoperoxidase and lysozyme also exhibit distinct antibacterial activities (Pakkanen and Aalto, 1997; Floris *et al*., 2003; Benkerroum, 2010).

The major proteins present in whey are *β*-lactoglobulin (50%) and *α*-lactalbumin (20%). Bovine *β*-lactoglobulin is a globular protein of a molar mass (Mw) of 18.3 kDa, existing mainly as a dimer at neutral pH. Bovine *α*-lactalbumin is a small compact globular protein with a Mw of 14.2 kDa. *β*-

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lactoglobulin and *α*-lactalbumin possess a variety of such useful functional characteristics as viscosity, gelation, foaming, solubility and emulsification that have rendered them as proper choices in the formulation of modern foods and beverages (Chatterton *et al*., 2006). Although proteolytic digestion is not desirable for functional characteristics, however it produces peptide fragments of various bioactivities. It has been reported that the various peptides derived from the proteolytic digestion of *β*-lactoglobulin and from *α*-lactalbumin have inhibitory effects against the angiotensin-converting enzyme. Antimicrobial, immunomodulating, opioid and hypocholesterolemic activities have also been documented (Brew and Grobler, 1992; Pellegrini *et al*., 2001; Sternhagen and Allen, 2001; FitzGerald *et al*., 2004; Korhonen and Pihlanto, 2004; Hernández-Ledesma *et al*., 2008; Park, 2009).

Antibacterial peptides, lactoferrin (Bellamy *et al*., 1992), *α*-lactalbumin (Pellegrini *et al*., 1999) and *β*-lactoglobulin (Pihlanto-Leppa *et al*., 1999; Pellegrini *et al*., 2001; and El-Zahar *et al*., 2004) lately have been derived from bovine whey proteins.

Native bovine *β*-lactoglobulin and *α*-lactalbumin are resistant to enzymatic proteolysis but, their extensive hydrolysis has been observed through application of a relatively long incubation periods (44 hours) (Schmidt and Poll, 1991; Guo *et al.,* 1995, Dalasgaard *et al*., 2008). Release of antibacterial peptides from whey proteins is typically achieved using such enzymes as pepsin, trypsin, and chymosin. However, to the best of our knowledge, no study has been carried out to identify antibacterial properties of Plasmin-Digests of *β*-lactoglobulin (PDβ) and of *α*-lactalbumin (PDα) against pathogenic as well as probiotic bacteria.

Plasmin is the most important endogenous proteinase associated with casein in bovine milk. Plasmin is a heat-stable alkaline serine proteinase, optimally active at a pH of about 7.5 and a temperature of 37°C. Therefore, plasmin is not inactivated through pasteurization with proteolysis of milk proteins being continued during dairy product manufacture and storage. Some such diseases as mastitis are associated with increased plasmin level and this enzyme damages milk proteins by breaking the original large protein chains into smaller peptides (Fox, 1991, Thompson *et al*., 2009). Although plasmin is a natural endogenous proteinase in bovine milk, the effect of this enzyme on antibacterial properties of milk proteins and especially on *β*-lactoglobulin and on *α*-lactalbumin has not been reported.

The objective of the present study was to evaluate the antibacterial effects of PDβ, and of PDα against pathogenic (*E. coli* and *S. aureus*) and probiotic (*L. casei* and *L. acidophilus*) bacteria and to make a comparison with nisin (a bacteriocin produced by *Lactococcus lactis*) as regards the antibacterial potentials. The study is also intended to determine the changes in the growth curves and in the plate count confirmation of pathogenic and probiotic bacteria in the presence of either one of PDβ, PDα or nisin.

MATERIALS AND METHODS

Bovine *β*-lactoglobulin, *α*-lactalbumin, nisin and bovine plasmin (EC Number 3.4.21.7) were supplied from Sigma-Aldrich Chemie GmbH (Munich, Germany). Sodium dihydrogen phosphate, Sodium monohydrogen phosphate, Trifluoroacetic Acid (TFA), Acetonitrile (grade A), Brain-Heart Infusion Agar (BHIA), Brain-Heart Infusion Broth (BHIB), MRS Agar (Man, Rogosa and Sharpe Agar), and MRS Broth (Man, Rogosa and Sharpe Broth) were obtained from Merck (Darmstadt, Germany). Cultures of *Escherichia coli* (PTCC 1399*)* and *Staphylococcus aureus* (PTCC 1431) came from the Iranian Research Organization for Science and Technology Company (IROST) in Tehran.

Cultures of *Lactobacillus acidophilus* (DSMZ 1643) and *Lactobacillus casei* (DSMZ 1608) were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen Germany company.

Enzymatic Hydrolysis

The bovine *β*-lactoglobulin and *α*-lactalbumin with concentrations of 3 mg ml^{-1} were prepared in 10 mM phosphate buffer pH 6.8. Bovine plasmin was added to the aliquots of bovine *β*-lactoglobulin and to *α*-lactalbumin substrate proteins at an enzyme: substrate ratio of 1:150 (v/v). Enzymatic hydrolysis was implemented through incubation at 30°C for 44 hours.

Separation of Peptides in the Plasmin Hydrolysates

The separation of plasmin digest of proteins was similar to that in the published method (Dalasgaard *et al*., 2008). Fractionation of the plasmin digest of bovine *β*-lactoglobulin and of *α*-lactalbumin were performed through Unicam crystal 200 series HPLC (Cambridge, United Kingdon). Aliquots of the PDβ and PDα samples were injected onto a C18 reversed phase column (15 × 2.1 mm, 5-µm particle size). The solvents consisted of: (A) 0.1% TFA in water and (B) 80% acetonitrile, 0.1% TFA. A linear gradient of solvent B with a time schedule of 2 to 10 minutes: 40%, 15 minutes: 50%, 45 to 50 minutes: 100% of solvent B were applied. The UV detector recorded results at 241 nm. The RP-HPLC separations were repeated in a number of three replicates.

Antibacterial Assay

β-lactoglobulin, *α*-lactalbumin, plasmin, nisin, PDβ and PDα were tested for antibacterial activity against pathogenic and as well against probiotic bacteria. For assaying the overnight of bacteria, every culture was diluted to approximately 10^6 cell ml^{-1}. To each sterile Eppendorf vial, 450 µl of either BHI or MRS broth, 50 µl of the mentioned compound, along with 10 µl of overnight cultured bacteria were added. Control sample contained 50µL of 20 mM phosphate buffer in place of peptide solution and while blank contained phosphate buffer in place of any of the peptide solutions, and as well the overnight cultured bacteria. The plasmin antibacterial experiment was performed for different concentrations of plasmin solution (enzyme: buffer ratio of 1:15 (v/v) to 1:300 (v/v)) in place of the peptide solution. Antibacterial test of nisin (from *Lactococcus lactis*) was performed by adding nisin powder to 0.02M hydrochloric acid and having it centrifuged at 7,000g for 10 minutes at 4°C. For sterilization, the solution was filtered through a 0.2 µm filter. The vials were incubated at 37°C for 18 hours (36 hours for probiotic bacteria).

Optical density was assessed at 620 nm applying Cecil Spectrophotometer (Cecile 7400 UV-Visible, Cambridge, England) for all the samples. The experiments were repeated three times for each sample.

Minimum Inhibitory Concentration (MIC) of Antibacterial Compounds

Minimum Inhibitory Concentration (MIC) assays were carried out only for the antibacterial compounds. The MIC for these compounds was defined as the lowest concentration of antibacterial compound that resulted in no increase of absorbance at 620 nm following incubation (McCann *et al.,* 2006).

For an assay of the overnight of any bacteria, the culture was diluted to approximately 10^6 cell ml^{-1}. To each sterile Eppendorf vial, 450 µl of either BHI or MRS broth, different concentrations of antibacterial compound (ranging from 0 to 600 ppm), and 10µl of overnight cultured bacteria were added. Control sample contained different concentrations of phosphate buffer in place of the antibacterial compound.

The vials were incubated at 37°C for 18 hours (36 hours for probiotic bacteria). Optical density was read at 620 nm for all the samples. The experiments were repeated thrice for each sample.

Effect of Antibacterial Compounds on Growth Curves and Plate Count Confirmation of Bacteria

For an assay of the overnight of any bacteria, the culture was diluted to approximately 10^6 cell ml^{-1}. To each sterile Eppendorf vial, either BHI or MRS broth, along with 10 µl of overnight cultured bacteria were added. The antibacterial compound was added in different concentrations (MIC concentrations, 0.5 and 0.25 MIC concentrations) (Table 1). Control experiment contained 20 mM of phosphate buffer in place of antibacterial solution. The vials were incubated at 37°C. Optical density was measured at 620 nm every two hours and over 24 hours time for pathogenic bacteria and every two hours over 48 hours time for probiotic bacteria. For plate count test, at each incubation period, a 1 ml sample was taken, diluted, and plated onto either BHI or MRS agar. These plates were incubated at 37°C for either 24 or 48 hours and then all the plates were read by the colony counter (Colony Star Funke Gerber, Germany).

RESULTS AND DISCUSSION

Evaluation the Chromatograms of the Hydrolysed Proteins

Digestion of *β*-lactoglobulin and *α*-lactalbumin by indigenous milk protease plasmin after 44 hours of incubation yielded several peaks, as separated through RP-HPLC. The chromatogram of *β*-lactoglobulin was divided into six distinct peaks with retention times of 10.4-, 12.6-, 13.8-, 14.4-, 15.9- and 16.6-minute (chromatogram not shown). Also, the chromatogram of *α*-lactalbumin was divided into eight distinct peaks with retention times of 10.3-, 11.8-, 14.1-, 15-, 16.1- 16.6- 17.2- and 18.1-minute (chromatogram not shown). The resulting chromatograms revealed that *β*-lactoglobulin and *α*-lactalbumin were hydrolyzed by plasmin when at 30°C for 44 hours. In these chromatograms, every peak represented the presence of one hydrolyzed peptide. These findings differ from those of the previous studies stating that *β*-lactoglobulin and *α*-lactalbumin were not degraded by plasmin (Chen and Ledford, 1971; Fox, 1991). However, our findings were in agreement with those of Dalasgaard *et al*. (2008), who reported extensive hydrolysis of these whey proteins over a relatively long incubation period (44 hours).

Evaluation of Antimicrobial Activity

In the present study the antibacterial activities of *β*-lactoglobulin, *α*-lactalbumin, Plasmin, PDβ, PDα as well as nisin were tested against *E.coli*, *S.aureus*, *L.case*i and *L.acidophilus*. Although *β*-lactoglobulin, *α*-lactalbumin and Plasmin exhibited no antibacterial effect against Gram-positive and Gram-negative bacteria, PDβ, PDα and nisin did demonstrate antimicrobial activity against all the target bacteria. Nisin, as a bacteriocin, was employed for an evaluation of the antibacterial properties of PDβ and PDα. Nisin revealed antibacterial activity against all the Gram-positive bacteria, but this bacteriocin bore no inhibitory effect on Gram-negative bacteria. PDβ and PDα which contained high levels of unknown peptides, revealed significant antibacterial properties against all the target bacteria (Table 2).

Although Farouk (1982) reported antibacterial activity of trypsin and chymotrypsin, they used higher enzyme concentrations.

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Table 1. Different concentrations (µg ml^{-1}) of antibacterial compound were used to evaluate the growth curve changes and growth inhibition curve.

Sample	*E. coli*	*S. aureus*	*L. casei*	*L. acidophilus*
PDβ	MIC (50 µg ml^{-1})	MIC (20 µg ml^{-1})	MIC (15 µg ml^{-1})	MIC (15 µg ml^{-1})
PDα	MIC (40 µg ml^{-1})	MIC (18 µg ml^{-1})	MIC (12 µg ml^{-1})	MIC (12 µg ml^{-1})
Nisin	MIC (550 µg ml^{-1})	MIC (3 µg ml^{-1})	MIC (2 µg ml^{-1})	MIC (2 µg ml^{-1})

Table 2. Numbers (log10 cfu ml^{-1}) of surviving bacteria following exposure to antibacterial compounds.

Samples[a]	Numbers (log10 cfu ml^{-1}) of surviving bacteria			
	E. coli	*S. aureus*	*L. casei*	*L.acidophilus*
Nisin	8.89 ±0.16	ND[b]	ND	ND
PDβ	ND	ND	ND	ND
PDα	ND	ND	ND	ND
Control	8.89 ±0.16	8.95±0.14	9.61±0.14	10±0.14

[a] PD*β*= Plasmin-Digest of *β*-lactoglobulin; PDα= Plasmin-Digest of *α*-lactalbumin, Control= Control sample, containing phosphate buffer in place of antibacterial compounds.
[b] Not Determined, as no growth was observed following incubation.

Although intact *β*-lactoglobulin and *α*-lactalbumin had no antibacterial effect on Gram-positive and Gram-negative bacteria, their plasmin digest, namely PDβ and PDα did show antibacterial activity against the bacteria tested. The β-lactoglobulin and α-lactalbumin chromatograms showed that following Plasmin Digestion, PDβ and PDα had hydrolyzed peptides and these peptides might have revealed antibacterial activity. To the best of our knowledge, there is no report regarding antibacterial potential of PDβ and PDα. However, Pellegrini *et al*. (1999) and as well Pellegrini *et al*. (2001), reported that peptides produced from proteolytic digestion of *β*-lactoglobulin and *α*-lactalbumin rendered antibacterial activity against the bacteria tested.

In agreement with the results obtained in the present study, Pihlanto-Leppala *et al*. (1999), reported that undigested *β*-lactoglobulin and *α*-lactalbumin exhibited no inhibitory activity against *E. coli* JM103 at a high concentration, while proteolytically digested *β*-lactoglobulin and *α*-lactalbumin inhibited the activity of the tested bacteria. The findings in the present study are not in agreement with those of Chaneton *et al*. (2011), who reported that intact *β*-lactoglobulin isolated from fresh milk inhibited the growth of *S.aureus* and *St.uberis* but had no effect on *E. coli*. It should be noted that they had tested antibacterial activity of *β*- lactoglobulin against a lesser number of target bacteria as compared with that in the present study.

Determination of Minimum Inhibitory Concentration (MIC)

The respective MICs against target bacteria following the bacteria's exposure to the antibacterial compounds (PDβ, PDα and nisin) are presented in Table 3.

As can be observed, the MIC of PDβ and PDα peptides ranged from 12 to 20 µg ml^{-1} against the Gram-positive bacteria (*Staphylococcus aureus, Lactobacillus casei* and *Lactobacillus acidophilus*), while around 50 µg ml^{-1} against Gram-negative bacterium (*Escherichia coli*), indicating that PDβ and PDα are active against all the tested bacteria. The MIC results for the PDβ and PDα indicated that these compounds' high antibacterial potential. The observation is explainable by the presence of antibacterial peptides and as well by the synergism among the peptides within PDβ and PDα.

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Table 3. Minimum Inhibitory Concentration (MIC) of antibacterial compounds against the selected bacteria

	MIC (µg ml^{-1})			
Samples	*E. coli*	*S. aureus*	*L. casei*	*L. acidophilus*
PDβ	50	20	15	15
PDα	40	18	12	12
Nisin	550	3	2	2

Both PDβ and PDα had higher MIC values for Gram-negative as compared with Gram-positive bacteria (about twice as high). The higher resistance of Gram-negative bacteria might be attributed to the complexity of their cell membrane structure as their cell wall contains an outer membrane consisting of lipopolysaccharide, phospholipid, lipoprotein, and protein in addition to a cytoplasmic membrane, all of which add strength to a Gram-negative bacterium's cell membrance (Hancock and Lehrer, 1998).

In contrast, nisin exhibited a relatively low MIC ranging from 2 to 3 µg ml^{-1} against Gram-positive bacteria. Nisin MIC against Gram-negative bacteria, *E.coli* (550 µg ml^{-1}) was very high. The inactivity of nisin against Gram-negative bacterium results from its relatively large size, preventing it from easily penetrating the outer membrane of the Gram-negative cell wall (Heike and Sahl, 2000). Nisin MIC values against Gram-negative bacterium were 11 times those of PDβ and PDα, but these MIC values against Gram-positive bacteria were 5 times lesser than those of PDβ and PDα. This finding is consistent with Kordel *et al*. (1989); Ganzle *et al*. (1999) and Boziaris and Adams (1999) who reported that Gram-negative bacteria were highly resistant to bacteriocins.

Effect of Antibacterial Compounds on the Growth Curves of Bacteria

The growth curves of pathogenic (*E. coli* and *S. aureus*) and of probiotic bacteria (*L. casei and L. acidophilus*) were obtained as recorded by measuring the optical density over either 24 or 48 hours. The growth curve of *E. coli* and *S. aureus* in the presence of PDβ, PDα and nisin is shown in Figure 1. Although the control had a lag phase in the first 2 hours, the growth curve of *E. coli* and *S. aureus* in the presence of the PDβ, PDα and nisin showed a longer lag phase in the first 3 to 10 hours. 2 hours past, OD620 for the control increased at a faster rate and then kept stable. OD620 for the treated samples increased at a lesser than the control and remained steady at a low value. The difference between OD620 for the control and those for the treated samples indicated that the growth of *E. coli* and *S. aureus* had been inhibited by the presence of nisin and especially PDβ and PDα's presence.

The growth curve for *L. casei* and *L. acidophilus* in the presence of PDβ, PDα, and nisin is shown in Figure 2. The control showed a lag phase for the first 2 to 4 hours, and while the treated samples showing a lag phase within the first 6 to 16 hours. 2 hours past, OD620 increased for the control group at a faster rate and was then kept stable. OD620 of the treated samples increased at a slower rate than that of the control group and remaining then steady at a low value. The difference between OD620 values for the control and treated samples indicated that the growth of *L. casei* and *L. acidophilus* had been inhibited by the presence of PDβ and PDα and especially nisin. The results obtained indicated that the effect of different concentrations of PD*β,* PDα and nisin on the lag phase and on the maximum OD620 of *E. coli, S. aureus, L. casei* and *L. acidophilus,* compared with their controls, were statistically significant ($P< 0.05$).

The growth curves of bacteria showed similar patterns in the presence of PDβ, PDα

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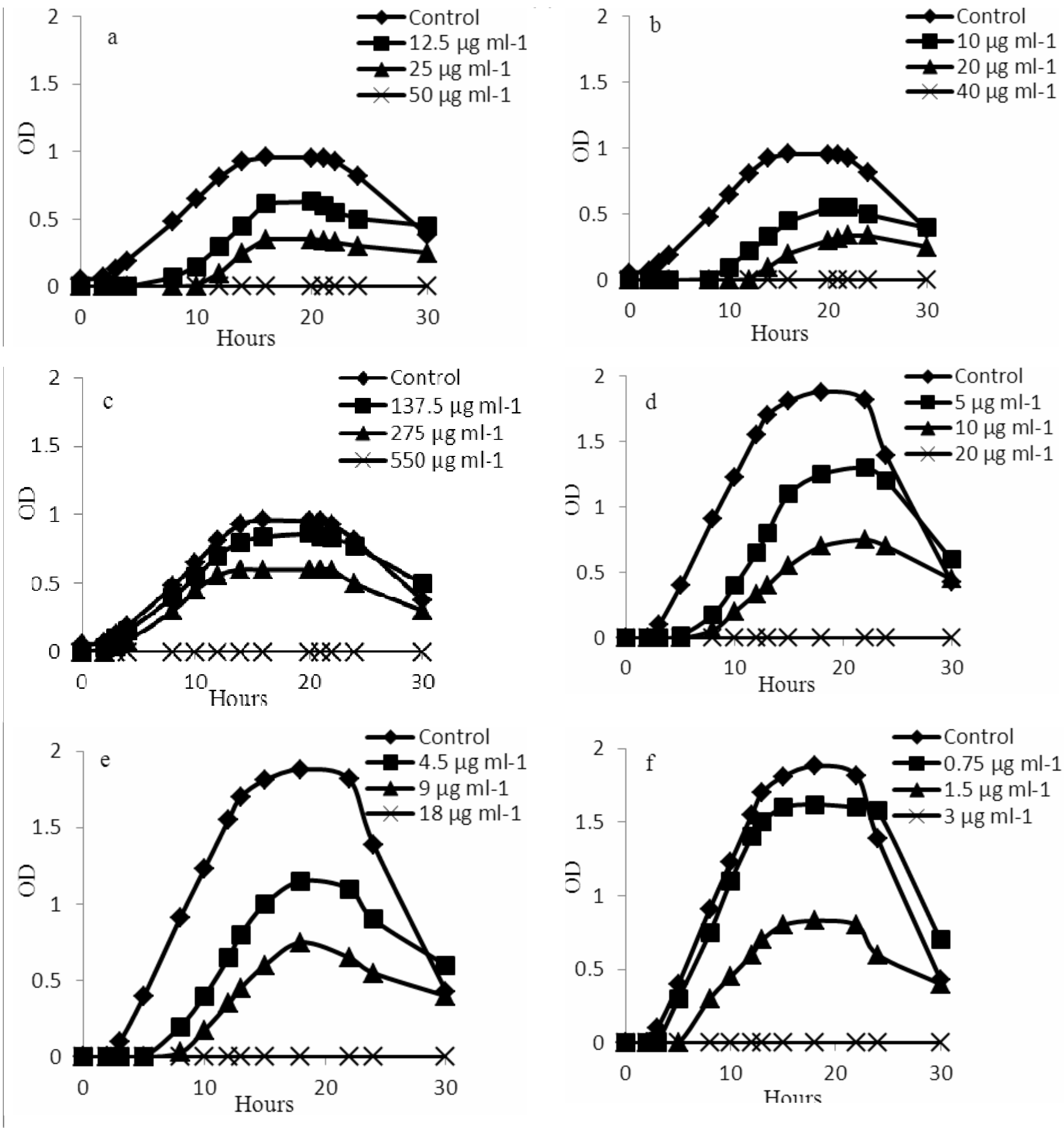

Figure1. Growth curves of patterns' assay: Antibacterial effect of Plasmin-Digest of *β*-lactoglobulin (PDβ) on *Escherichia coli* (a) on *Staphylococcus aureus* (d); antibacterial effect of Plasmin-Digest of *α*-lactalbumin (PDα) on *Escherichia coli* (b) on *Staphylococcus aureus* (e), nisin antimicrobial effect on *Escherichia coli* (c) and on *Staphylococcus aureus* (f) over time, and at different concentrations. Controls contain no antibacterial compounds.

and nisin. The inhibitory effects of PDβ, PDα and nisin on the tested bacteria dependent on concentration, higher concentrations rendering stronger antibacterial activity. These findings are consistent with the findings of Vongsawasdi *et al*. (2012), who demonstrated that inhibition of *S. aureus* increased as nisin concentration and its incubating time increased.

The growth curves for the bacteria in the presence of these compounds showed longer lag phases as compared with controls. The growth curves of bacteria showed a maximum difference in optical density between treated samples and control during

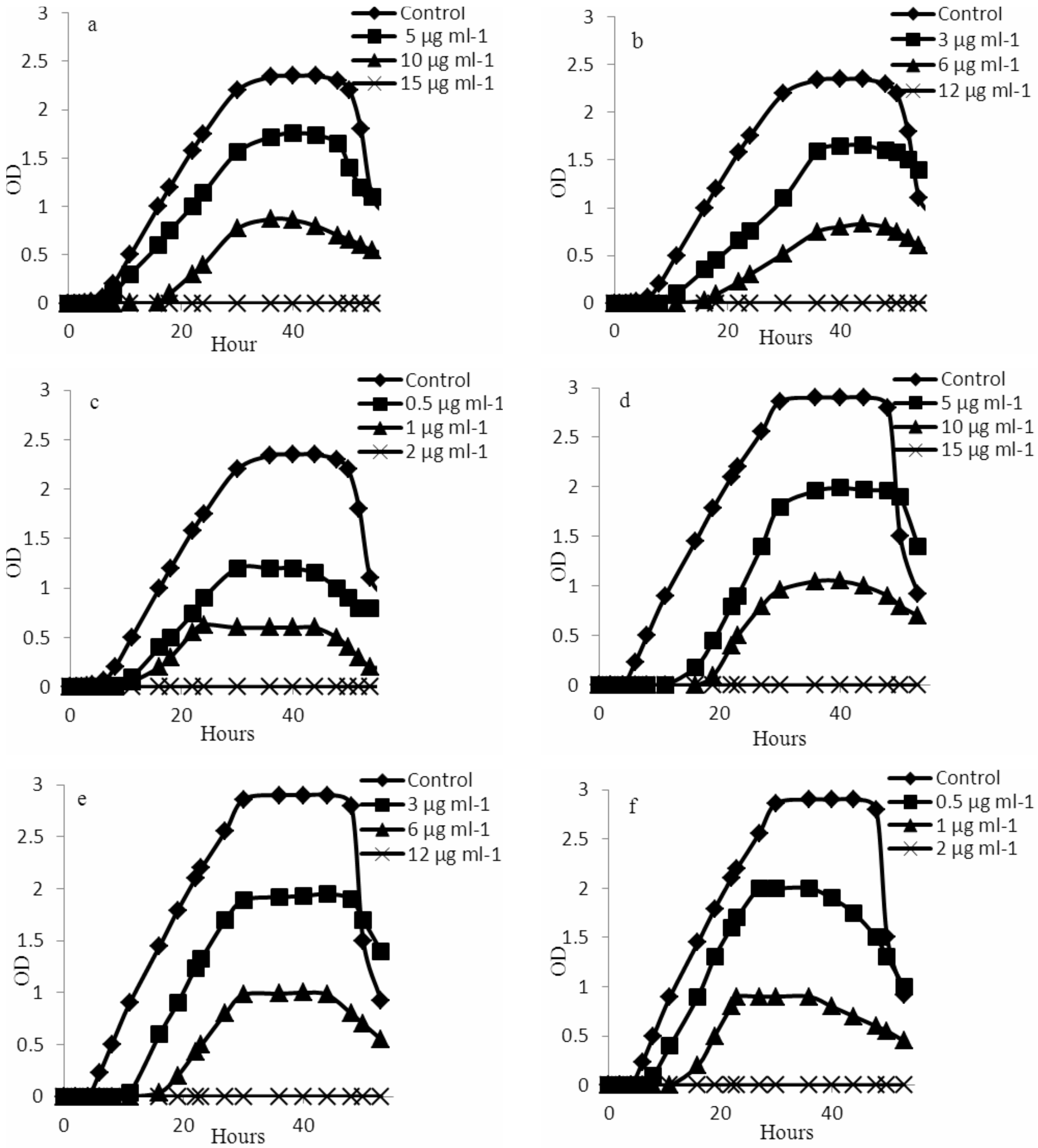

Figure2. Growth curves of patterns' assay: Antimicrobial effect of Plasmin-Digest of *β*-lactoglobulin (PDβ) on *Lactobacillus casei* (a) on *Lactobacillus acidophilus* (d); antimicrobial effect of Plasmin-Digest of *α*-lactalbumin (PDα) on *Lactobacillus casei* (b) and on *Lactobacillus acidophilus* (e), antimicrobial effect of nisin on *Lactobacillus casei* (c) and on *Lactobacillus acidophilus* (f) over time, and at different concentrations. Controls contain no antibacterial peptide.

the log phase. The differences in optical densities between treated samples and control, during the stationary and death phases were less than those for the log phase. These results indicate that a maximum sensitivity of bacteria to PDβ, PDα and to nisin occurred during their log phases.

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Plate Count for Bacteria in the Presence of Antibacterial Compounds

Figure 3 shows the plate counts for *E. coli* and *S. aureus* in the presence of PDβ, PDα and nisin. The results show that *E. coli* and *S. aureus* were affected by PDβ, PDα and nisin by maximum 3.19 and 3.97 log cfu ml^{-1} differences respectively, over the control and after a passage of 16 hours (MIC concentration). *L. casei* and *L. acidophilus* showed respective maximum differences of 5.1 and 4.86 log cfu ml^{-1} between control *vs.* treated samples after 36 hours past (MIC concentration) (Figure 4). These results

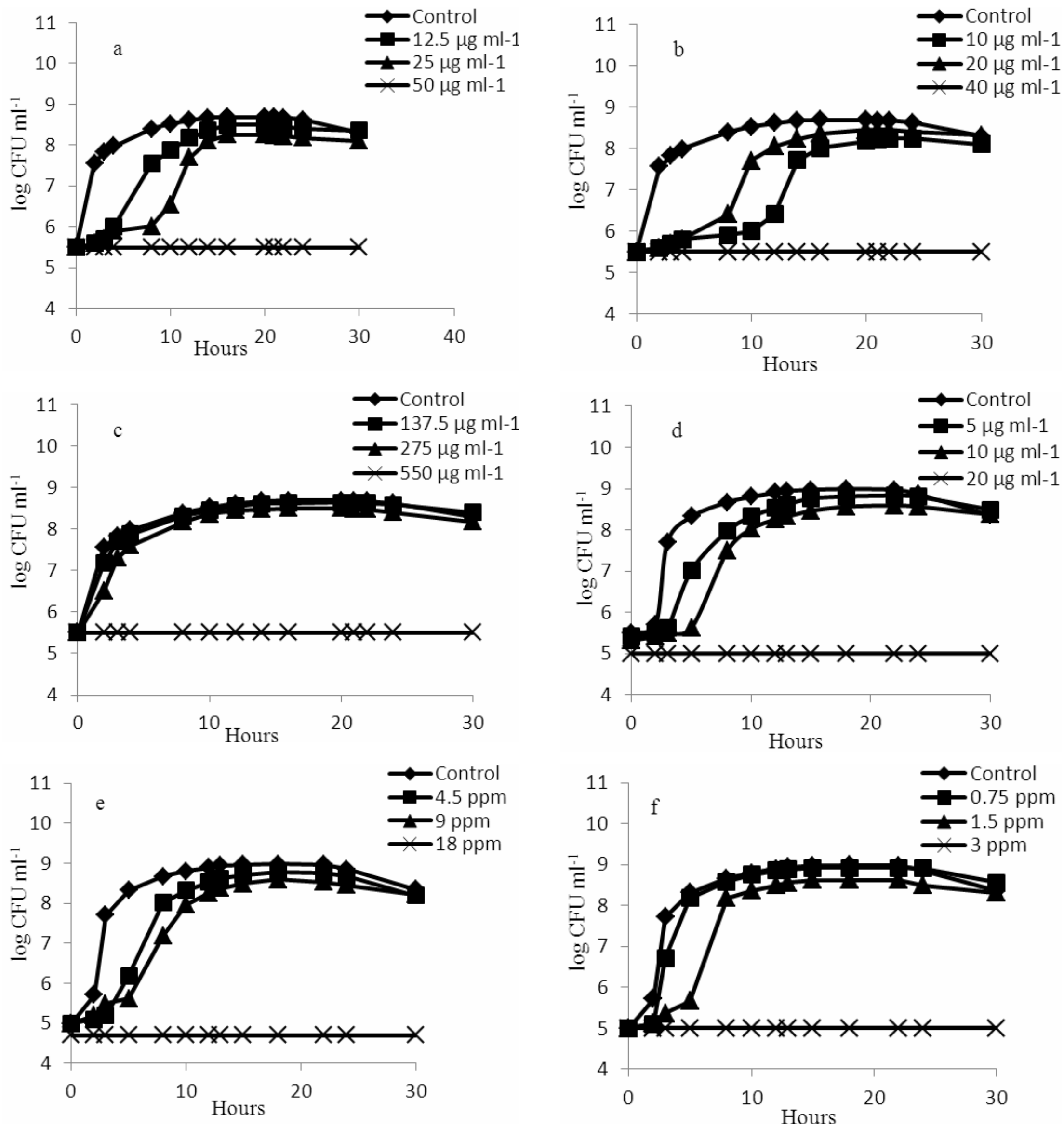

Figure3. Growth inhibition curves: Effect of Plasmin-Digest of *β*-lactoglobulin (PDβ) on numbers (log10 cfu ml^{-1}) of surviving *Escherichia coli* (a) on *Staphylococcus aureus* (d); effect of Plasmin-Digest of *α*-lactalbumin (PDα) on numbers (log10 cfu ml^{-1}) of surviving *Escherichia coli* (b) on *Staphylococcus aureus* (e), and the effect of nisin on numbers (log10 cfu ml^{-1}) of surviving *Escherichia coli* (c) and on *Staphylococcus aureus* (f) over time, and at different concentrations. Controls contain no antibacterial peptide.

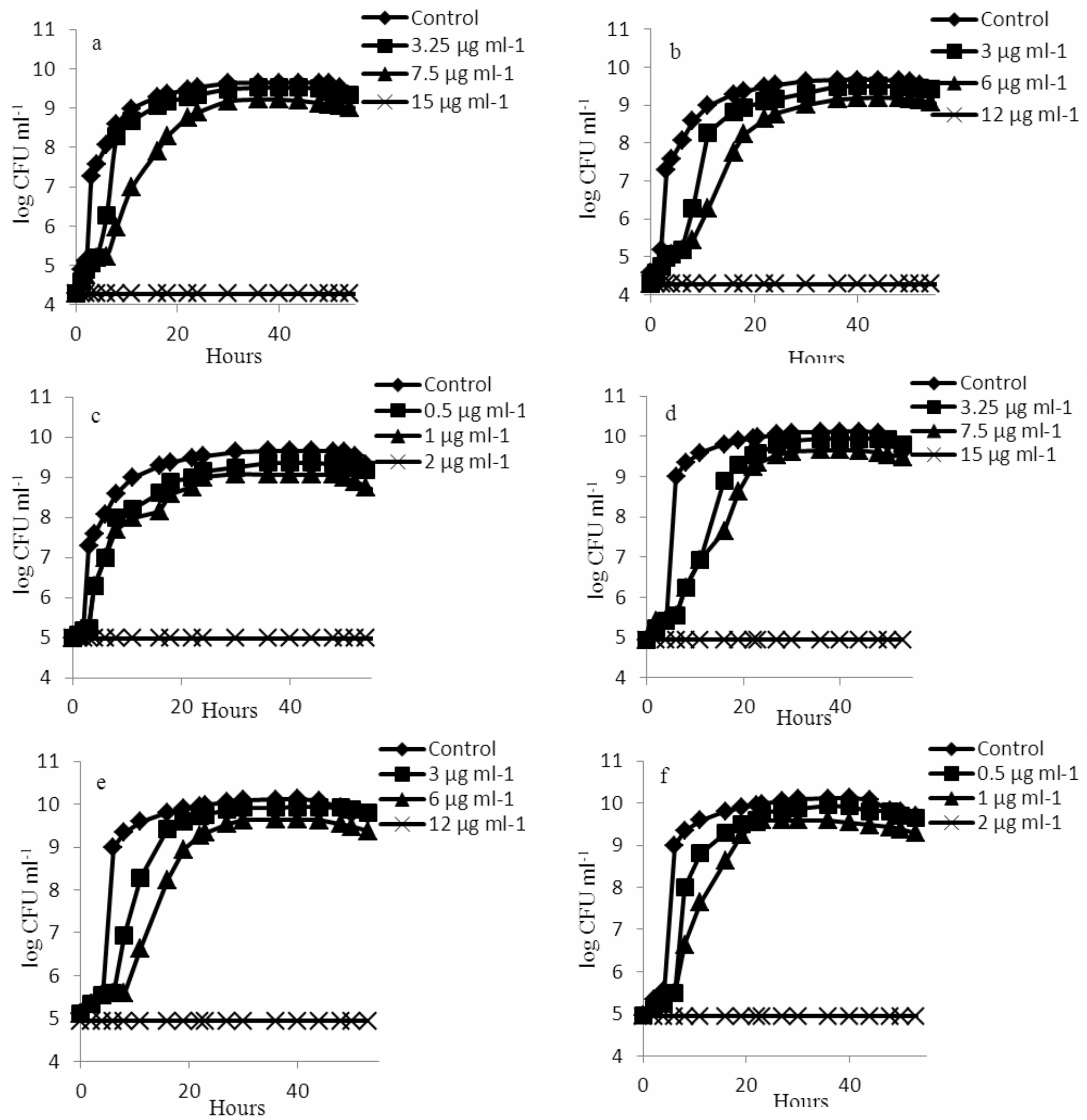

Figure4. Growth inhibition curves: Effect of Plasmin-Digest of *β*-lactoglobulin (PDβ) on numbers (log10 cfu ml^{-1}) of surviving *Lactobacillus casei* (a) on *Lactobacillus acidophilus* (d); effect of Plasmin-Digest of *α*-lactalbumin (PDα) on numbers (log10 cfu ml^{-1}) of surviving *Lactobacillus casei* (b) on *Lactobacillus acidophilus* (e), and the effect of nisin on numbers (log10 cfu ml^{-1}) of surviving *Lactobacillus casei* (c), and on *Lactobacillus acidophilus* (f) over time, and at different concentrations. Controls contain no antibacterial peptide.

indicated that maximum difference in log cfu ml^{-1} between treated samples and control during log phase for probiotic bacteria was higher than that for pathogenic bacteria. This could be because control group of probiotic bacteria, in the log phase, can obtain a higher log cfu ml^{-1} as compared with the control group related to the pathogenic bacteria.

Although pathogenic and probiotic bacteria, in the presence of PDβ, PDα and nisin, had longer lag phases than the control, the numbers of bacteria in the lag phases showed limited uariations. In the log phase, the plate count *E. coli* and *S. aureus* in the presence of nisin showed differences of 0.18 and 2.65 respectively from the controls (less than the MIC concentration). In the presence of PDβ, *E.coli* and *S.aureus* were different from control by 2.21 and 2.60, respectively, (less than the MIC concentration). In the presence of PDα, *E. coli* and *S. aureus* had differences of 2.52 and 2.70 log cfu ml^{-1}, respectively, over the controls (less than the MIC concentration). These differences in the stationary phase for treated samples were less than those for the log phase. The death phase showed the least difference in the number of bacteria from the control. These results indicate that nisin in its less than MIC concentration could not effectively reduce log cfu ml^{-1} of *E.coli.*

In the log phase, the plate counts of *L. casei* and *L. acidophilus* in the presence of nisin differed by of 1.64 and 2.3, respectively, as compared with the controls (less than the MIC concentrations). In the presence of PDβ, *L. casei* and *L. acidophilus* differed by 2.82 and 3.1, respectively, from their controls. These differences in the presence of PDα were 3.14 and 2.95 log cfu ml^{-1} respectively and as compared with the control. The difference in the stationary phase for the treated samples was less than that for the log phase. The death phase exhibited the least difference in the number of bacteria as compared with control.

Growth inhibition curves also showed the maximum difference in log cfu ml^{-1} between treated samples and their control during the log phase. As a result, changes in the bacterial growth curves were consistent with the changes in the growth inhibition curves. There was a statistically significant difference observed, in mean number, between bacteria surviving in the presence of different concentrations of PDβ, PDα and nisin *vs.* bacteria present in the control samples ($P< 0.05$).

In the current study, the effect of PDβ and PDα on pathogenic and on probiotic bacteria was evaluated, and the results compared with when nisin used. The growth curves of bacteria revealed similar patterns in the presence of PDβ, PDα and nisin. All tested bacteria exhibited the most sensivity to PDβ and PDα in the log phase. As a result, it is recommended that one should use them in the log phase for stopping pathogenic bacteria survival in a medium.

The effect of PDβ and PDα on pathogenic bacteria revealed that these compounds benefit from a good potential for increasing food microbial safety. Therefore, PDβ and PDα have great potential as natural food additives in the food chain. In the meantime that PDβ and PDα are effective in controlling pathogenic bacteria, these compounds could also effectively control probiotic bacteria too. Most probiotic bacteria strains are among the Gram-positive ones, and PDβ and PDα are more effective on stopping the survival of Gram-positive bacteria. The viability of probiotic microorganisms in the final product until the time of its consumption is their most important qualitative parameter (Mortazavian and Sohrabvandi, 2006). The effect of PDβ and PDα on pathogenic bacteria, increases the safety of raw milk and dairy products, however, the effect of these compounds on probiotic bacteria is not desirable. Thus, further evaluation of the effect of PDβ and PDα on the viability of probiotic bacteria in the final product and under different conditions is necessary.

CONCLUSIONS

Results of the present study show that antimicrobial activity can be influenced by PDβ and PDα. This result increases understandings of *β*-lactoglobulin and *α*-lactalbumin and reveals that bactericidal peptides can be produced by the proteolytic digestion of *β*-Lactoglobulin and *α*-lactalbumin from milk protease plasmin. Throughout the present study, PDβ and PDα with a potent inhibition against either of Gram-positive or Gram-negative bacteria were scrutinized. It is expected that PDβ and PDα might have a potentially valuable role as food additives and as well in strengthening the immune system of the

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host. Some of the Gram-positive bacteria in the study were probiotic, so the effect of PDβ and PDα on probiotic products should be further stressed. While PDβ and PDα are effective against Gram-negative bacteria, nisin is more effective against Gram-positive ones. Thus the application of a mixture of antibacterial peptides along with nisin might enhance the quality in a food product

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بتا لاکتوگلوبولین و آلفالاکتوآلبومین هیدرولیز شده به عنوان منبع پپتیدهای ضد میکروبی

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چکیده

در این تحقیق وجود فعالیت ضد باکتریایی در بتا لاکتوگلوبولین و آلفالاکتوآلبومین گاوی هیدرولیز شده مورد بررسی قرار گرفت. پپتیدهای موجود در بتا لاکتوگلوبولین و آلفالاکتوآلبومین گاوی

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هیدرولیز شده با آنزیم پلاسمین به وسیله دستگاه RP-HPLC جداسازی شد. فعالیت ضد باکتریایی بتا لاکتوگلوبولین و آلفالاکتوآلبومین، نیسین، پلاسمین، بتا لاکتوگلوبولین و آلفالاکتوآلبومین هیدرولیز شده در برابر باکتری های بیماریزا (اشرشیاکلی و استافیلوکوکوس اورئوس) و سلامتی بخش (لاکتوباسیلوس کازئی و لاکتوباسیلوس اسیدوفیلوس) در آزمایشگاه ارزیابی شد. اگرچه بتا لاکتوگلوبولین و آلفالاکتوآلبومین و پلاسمین فعالیت ضد باکتریایی در برابر باکتری های هدف نشان ندادند، بتا لاکتوگلوبولین هیدرولیز شده، آلفالاکتوآلبومین هیدرولیز شده و نیسین در برابر باکتری های هدف خاصیت ضد باکتریایی داشتند. در مرحله بعد حداقل غلظت بازدارندگی (MIC) ترکیبات با خاصیت ضد باکتریایی در برابر باکتری های هدف تعیین شد. نتایج حاصل مشخص کرد، حداقل غلظت بازدارندگی این ترکیبات و نیسین در برابر باکتری های گرم مثبت کمتر از باکتری های گرم منفی است. در این مطالعه تاثیر بتا لاکتوگلوبولین هیدرولیز شده، آلفالاکتوآلبومین هیدرولیز شده و نیسین بر منحنی رشد باکتری ها و شمارش کلی باکتری های زنده بررسی شد. نتایج حاصل مشخص کرد بتا لاکتوگلوبولین هیدرولیز شده، آلفالاکتوآلبومین هیدرولیز شده و نیسین اثر بازدارنده بر دوره کمون، ماکزیمم رشد و شمارش کلی باکتری های زنده باکتری های مورد آزمون دارند. بیشترین اثر بازدارندگی این ترکیبات در فاز رشد باکتری ها مشاهده گردید. اثر بازدارنگی این ترکیبات به غلظت آنها بستگی داشت، با افزایش غلظت، خاصیت ضد باکتریایی افزایش می یافت. بتا لاکتوگلوبولین هیدرولیز شده وآلفالاکتوآلبومین هیدرولیز شده در برابر باکتری های گرم منفی بسیار فعال تر از نیسین بودند ولی نیسین فعالیت بازدارندگی خوبی در برابر باکتری های گرم مثبت نشان می داد.