J. Food and Dairy Sci., Mansoura Univ., Vol. 3 (4): 225 - 236, 2012

ASSESSMENT OF THE ANTIMICROBIAL ACTIVITY OF BUFFALO'S MILK CASEIN PEPTIDES

Ammar, El-Tahra M. A. ; A. El-Shazly ; W. M. El-Sharoud and Samar A. Zalma
Dairy Department, Faculty of Agriculture, Mansoura University.

ABSTRACT

This study aimed to prepare peptides from buffalo milk's casein and to examine the antimicrobial effect of these casein peptides against diverse foodborne microorganisms. Casein was prepared by acid precipitation of buffalo's skimmed milk, and was found to consist of protein (83.19%), moisture (11.45%), ash (2.6%), fat (1.6%), and lactose (0.53%). Peptides were prepared from dried acid casein by proteolysis with chymosin and were dissolved to produce peptide concentrations of 10%, 20% 30%, and 40% (w/v%). These casein peptide preparations were assessed for their antimicrobial activity against Gram-positive, Gram-negative bacteria and yeasts. The antimicrobial effect of casein peptide solutions depended on the examined microorganism and the peptide concentration. *Salmonella* ser. Typhimurium, *Cronobacter sakazakii, Kluyveromyces marxianus*, and *Kodamaea ohmeri* could be variably inhibited by all casein peptide concentrations ranging from 10% to 40%. Whereas, *Escherichia coli* stains, and *Klebsiella pneumonia* could only be inhibited by solutions containing more than 10% casein peptides. *Shigella flexneri*, *Issatchenkia orientalis*, *Candida catenulate*, and *Clavispora lusitaniae* could only be inhibited by casein peptides concentrations higher than 20%. *Candida albicans* showed the highest resistance to casein peptides, as it could only be inhibited with the use of 40% casein peptides. These results showed that peptides prepared from acid casein by proteolysis with chymosin had an inhibitory effect against divers Gram-positive and Gram-negative bacteria and yeasts.
Keywords: Bioactive peptides, casein peptides, antimicrobial peptides, buffalo milk.

INTRODUCTION

It has been shown over the last few decades that food proteins could involve peptides of antimicrobial activity (Lopez-Exposito *et al*. 2006).These peptides have been prepared from various proteins in different food sources, including marine fish (Park *et al*. 1997), spinach (Segura *et al*. 1998), and eggs (Ibrahim *et al*. 2002). However, antimicrobial peptides derived from milk proteins have captured most of scientific interest (Floris *et al*. 2003, Lopez-Exposito *et al*. 2006). These antimicrobial peptides could be prepared from both whey proteins and caseins. For example, the lactoferricins peptides have been derived from bovine and human lactoferrin (Kitts and Weiler 2003, Wakabayashi *et al*. 2003). Other antibacterial peptides have been also identified in α_{s1}-casein (Lahov and Regelson 1996) and α_{s2}-casein (McCann *et al*. 2005).

Antimicrobial milk peptides were found to inhibit various pathogenic bacteria including pathogenic *Escherichia coli*, *Helicobacter sp*, *Listeria monocytogenes*, *Salmonella* and *Staphylococcus aureus*, in addition to yeasts and filamentous fungi. It has been also suggested that antimicrobial

peptides may modulate the intestinal microflora, when formed during milk digestion in the gastrointestinal tract (Shimizu 2004). Still, these antimicrobial peptides could have potential applications to the preservation of milk and dairy products by incorporating them as natural preservatives for inhibiting pathogenic and food spoilage microorganisms. Most of the relevant studies on antimicrobial milk peptides have utilized cow milk's proteins, with few studies using goat's and sheep's milks. To the best of our knowledge, there have been no previous reports on the use of buffalo's milk proteins for the preparation of antimicrobial peptides.

The present study was thus designed to prepare peptides from buffalo milk's casein and to examine the antimicrobial effect of those casein peptides against diverse foodborne microorganisms.

MATERIALS AND METHODS

Preparation of whole casein

Whole casein was prepared from skimmed buffalo's milk using the method described by Lahov and Regelson (1996) as follows. Twenty kilograms of raw skimmed buffalo's milk were slowly acidified to pH 4.6 by the addition of HCl (0.25N) at room temperature. Precipitated casein was separated by passing the resultant acid curd through wire mesh sieve, followed by washing with distilled water for 5 times. The resultant casein was air dried at room temperature for 72 h. Finally, it was milled, sieved and packed into sterile plastic containers.

Preparation of casein peptides

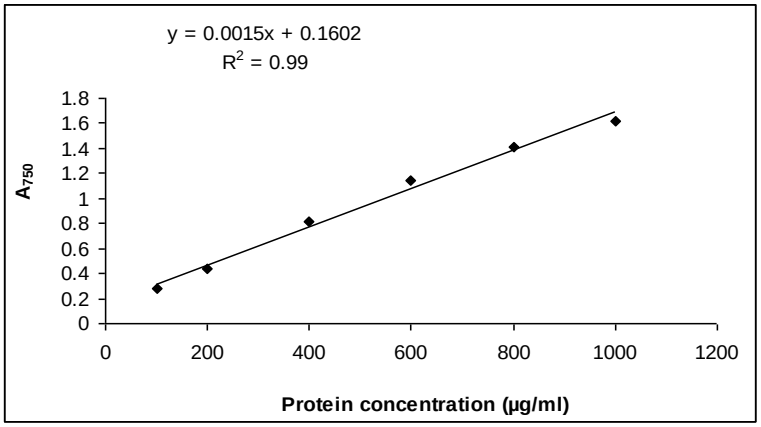
Dried buffalo's casein prepared as shown above was used to prepare a 1.7% (w/v) casein suspension by the addition of 25.5 g dried buffalo's casein into 1.5 lt pre-warmed distilled water. To facilitate the suspension of casein in water, NaOH (1/9 N) was gradually added up to pH 6.4 -7.0 with continuous agitation. After complete suspension of casein, chymosin was added to the solution at a concentration of 0.1 µg/ml, followed by incubation at 30ºC for 30 min to allow chymosin to hydrolyze casein. The resultant suspension was then heated at 80ºC for 3 min to inactivate the enzyme, followed by rapid cooling to room temperature. The suspension was further processed to separate the casein peptides as described by Lahov and Regelson (1996) as follows: Tri-chloro acetic acid (TCA) 8% (w/v %) was added to the suspension to give a final concentration of 2%. This was to precipitate para k-casein and casein-glucopeptides that were separated by centrifugation at 1370 *g* for 10 min at 4°C using SIGMA 4k15 centrifuge (SIGMA laborzentrifugen, DJB Labcare Ltd, England). Resultant supernatant was collected and treated with TCA 48% (w/v %) to give a final concentration of 12% to precipitate casein peptides that were then separated by centrifugation at 1370 *g* for 10 min at 2ºC. Supernatant was re-treated with TCA 48% (w/v %) to ensure the recovery of all casein peptides. Precipitates resulted from the previous two centrifugation runs were collected and dissolved in distilled water to final concentrations of 10%, 20%, and 30% (w/v %), and kept refrigerated at 6ºC till use as shown in Figure 1.

Figure 1: Preparation of peptides from whole dried casein.

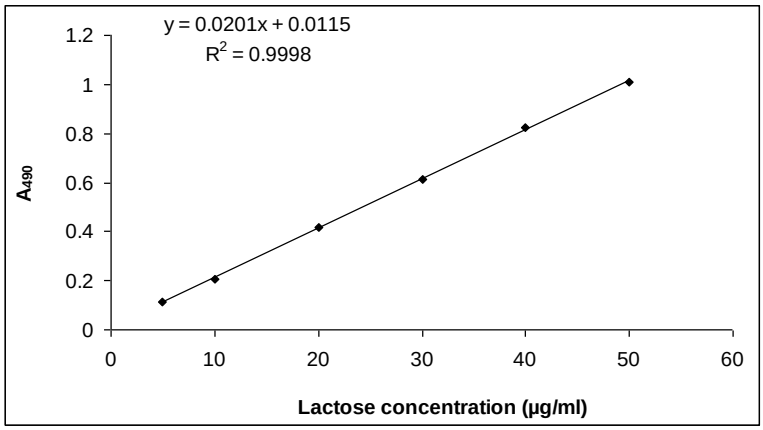
Chemical analysis

Moisture content, fat content, and ash content of skimmed buffalo's milk and whole dried casein were assessed as described by Ling (1963). Kjeldahl method was used to determine the total nitrogen, and thus protein content of skimmed buffalo's milk as described by Ling (1963). Whereas, the protein content of the whole dried casein was determined as described by Lowry *et al.* (1951) as follows: A sample of 0.1 g of whole dried casein was suspended in 100 ml distilled water pre-warmed to 40ºC with the gradual addition of NaOH (1/9 N) up to pH 6.4 – 7.0 with continuous agitation. One milliliter of the casein suspension was mixed well with 5 ml alkaline copper sulfate solution 0.5% (w/v %), followed by incubation for 5 min at the room temperature. An amount of 0.5 ml of diluted foline solution (1:1) was added and mixed well, followed by incubation for 30 min at room temperature, which resulted in the development of blue color. The intensity of this color was assessed by measuring light absorbance (A) at a wave length of 750 nm using the spectrophotometer (Jenway Ltd, Felsted, Dunmow, Essex, UK). Protein concentration was calculated by plugging the A_{750} reading into the line equation of a standard protein curve that related the concentration of bovine serum albumin (BSA) to A_{750}. To prepare the standard protein curve, 0.1 g of BSA was dissolved in 100 ml distilled water to provide a protein concentration of 1000 µg/ml. Different BSA concentrations were prepared from this solution by the addition of various amounts of distilled water. Resultant BSA preparations were processed as described above and A_{750} values were plotted against protein concentrations to produce the standard protein curve shown in Figure 2.A.

The lactose content of skimmed buffalo's milk and whole dried casein was determined using a colorimetric method described by Barnett and Abdel Tawab (1957) as follows: One gram sample of each of skimmed buffalo's milk and whole dried casein was added to 1 lt distilled water. To allow the suspension of dried casein, water pre-warmed to 40°C and NaOH were used as shown above. Six drops of phenol solution (80%) were added to 1 ml of each of the skimmed buffalo's milk and casein solutions, followed by the addition of 5 ml H_2SO_4 that resulted in the development of bulbar color. The intensity of this color was assessed by measuring light absorbance (A) at a wave length of 490 nm using the spectrophotometer. Lactose concentration was calculated by plugging the A_{490} value in the line equation of a standard lactose curve. This curve was prepared by dissolving 0.05 g lactose in 1 lt distilled water to provide a final concentration of (50 µg/ml). Different lactose concentrations were prepared from this solution by the addition of various amounts of distilled water. Resultant lactose preparations were analyzed as described above and A_{490} values were plotted against lactose concentrations to produce the standard curve presented in Figure 2.B.



(A)



(B)

Figure 2: Standard curves for protein (A) and lactose (B) photometric determinations

Assessment of antimicrobial activity of casein peptides

The antimicrobial activity of the casein peptides was tested against various microorganisms listed in Table 1 by using the agar diffusion technique described by Singh and Laxminaray (1973) and modified by Reinheimer *et al*. (1990) and Nassib *et al*. (2006). Examined cultures were grown in 10 ml tryptone soya broth (TSB) medium (Oxoid, Basingstoke, UK) at 37ºC for 24 h. Aliquots of 20 µL of each culture was swabbed by sterile cotton-tipped applicator on the surface of nutrient agar (NA), Sterile filter paper discs of 0.5cm in diameter were immersed separately into 10%, 20%, 30% casein peptide solutions and placed onto the surface of each inoculated NA plate. Finally, the NA plates were incubated at 37ºC for 24 h and the diameter of the inhibition zones around each filter paper disc was measured.

Table 1: Microbial cultures used in the assessment of antimicrobial activity of casein peptides

Microorganism	Source	Reference
Staphylococcus aureus FSMP262	Domiati cheese	El-Sharoud and Spano, (2008)
Salmonella ser. Typhimurium ATCC 14028	Laboratory culture	Kim and Thayer (1996)
Shigella flexneri ATCC 12022	Laboratory culture	American Type Culture Collection (ATCC)
Escherichia coli RH90	Laboratory culture	Lange *et al*. (1991)
Escherichia coli MC4100	Laboratory culture	Silhavy *et al*. (1984)
Cronobacter sakazakii CFS-FSMP 1511	Environmental, milk factory	El-Sharoud *et al*. (2009a)
Kelbsiella pneumoniae16	Yoghurt	Darwish (2011)
Candida albicans FSMPY4	Stored Domiati cheese	El-Sharoud *et al*. (2009b)
Issatchenkia orientalis FSMPY11	Kariesh cheese	El-Sharoud *et al*. (2009b)
Kluyveromyces marxianus FSMPY39	Matard cream	El-Sharoud *et al*. (2009b)
Candida catenulate FSMPY25	Kariesh cheese	El-Sharoud *et al*. (2009b)
Kodamaea ohmeri FSMPY10	Stored Domiati cheese	El-Sharoud *et al*. (2009b)
Clavispora lusitaniae FSMPY31	Kareish cheese	El-Sharoud *et al*. (2009b)

RESULTS AND DISCUSSION

The focus of this study was to prepare peptides from buffalo milk's casein and to examine the antimicrobial effect of those casein peptides against diverse foodborne microorganisms. To pursue this, whole casein was precipitated from raw skimmed buffalo's milk by slow acidification with HCl to pH 4.6. Then, precipitated casein was separated and washed with distilled water, followed by air drying at room temperature for 72 h. Dried casein was finally milled, sieved and packed into sterile plastic containers.

Table 2 shows the chemical characteristics of buffalo's skimmed milk and dried casein. It could be seen that buffalo's skimmed milk mainly consisted of moisture (91%), protein (3.49%), and lactose (3.9%), with minor amounts of fat (0.2%), and ash (0.66%). However, the major component of dried casein prepared from this buffalo's skimmed milk was protein that represented 83.19% followed by moisture (11.45%), ash (2.6%) and fat (1.6%) with lactose being a minor component representing 0.53%. The relative concentrations of most of these components in dried casein were comparable to those of commercial dried acid casein described by Morr (1982).

Table 2: Chemical characteristics of skimmed buffalo's milk and dried acid casein.

Characteristics	Skimmed buffalo's milk	Buffalo's dried casein
Moisture content (%)	91.0	11.45
Protein content (%)	3.49	83.19
Fat content (%)	0.2	1.6
Lactose content (%)	3.90	0.53
Ash content (%)	0.66	2.60
Titratable Acidity (TA%)	0.20	ND*
pH	6.6	ND*

***ND: Not determined.**

Dried acid casein prepared from buffalo's skimmed milk was used to prepare casein peptides. Briefly, whole dried casein was re-suspensed in distilled water to produce 1.7% (w/v) casein solution, followed by proteolysis with chymosin and a series of precipitation and separation steps as illustrated in Figure 1. Resultant casein peptides were dissolved in distilled water to produce peptide concentrations of 10%, 20% 30%, and 40% (w/v). These casein peptide preparations were assessed for their antimicrobial activity of the against Gram-positive, Gram-negative bacteria and yeasts by using the agar diffusion technique described by Singh and Laxminaray (1973) and modified by Reinheimer *et al*. (1990) and Nassib *et al*. (2006). In this technique, sterile filter paper discs impregnated with casein peptide solutions were placed onto the surface of nutrient agar, swabbed with each of the examined microorganisms. Plates were incubated at 37ºC for 24 h and the diameters of the inhibition zones around paper discs were measured.

Figure 3 shows the inhibition of the growth of 3 representative microorganisms by the application of paper discs impregnated with solutions containing casein peptide at 10%, 20%, 30%, and 40% (w/v%). Growth inhibition was indicated by the formation of clear zones around paper discs. The diameters of inhibition zones with various microorganisms and casein peptide concentrations are presented in Table 3. It could be seen in this table that the antimicrobial effect of casein peptide solutions depended on the examined microorganism and the peptide concentration. *Salmonella* ser. Typhimurium, *Cronobacter sakazakii, Kluyveromyces marxianus*, and *Kodamaea ohmeri* could be variably inhibited by all casein peptide concentrations ranging from 10% to 40%. Whereas, *E. coli* RH90, *E. coli* MC4199, and *Klebsiella pneumonia* could be only inhibited by solutions containing more than 10% casein peptides. There were 4 other microorganisms including *Shigella flexneri*, *Issatchenkia orientalis*, *Candida catenulate*, and *Clavispora lusitaniae* that could be only inhibited by casein peptides concentrations higher than 20%. *Candida albicans* showed the highest resistance to the antimicrobial effect of casein peptides, since it was not inhibited by solutions containing 10%, 20% or 30% casein peptide. This yeast could be only inhibited with the use of 40% casein peptides. Increasing the concentration of casein peptides was generally associated with increasing their antimicrobial action against the examined microorganisms. This was particularly noticed with *Salmonella* ser. Typhimurium and *kadamaea ohmeri,* where the diameters of the inhibition zones around paper discs impregnated with casein peptide solutions containing 40% were approximately two times the diameters of the inhibition zones produced with the use of a casein peptide concentration of 10% (Table 3).

Figure 3: Antimicrobial activity of casein peptides against 3 representative microorganisms including (A) *Staphylococcus aureus* (G+ bacteria), (B) *Klebsiella pneumonia* (G- bacteria), and (C) *Candida albicans* (Yeast)

Table 3: Diameters of inhibition zones around discs impregnated with casein peptides at different concentrations.

Microorganism	Diameter of inhibition zone including that of the paper disc (cm)			
	10% casein peptides	20% casein peptides	30% casein peptides	40% casein peptides
Gram-positive bacteria				
Staphylococcus aureus FSMP262	0.7	0.9	1.0	1.1
Gram-negative bacteria				
Salmonella ser. Typhimurium ATCC14028	0.6	0.8	0.8	1.2
Shigella flexneri ATCC12022	-	-	0.9	0.9
E. coli RH90	-	0.65	0.8	0.9
E. coli MC4100	-	0.6	0.8	0.8
Cronobacter sakazakii CFS-FSMP1511	0.7	0.8	0.8	0.9
*Klebsiella pneumoniae*16	-	0.7	0.8	0.9
Yeasts				
*Candida albicans*FSMPY4	-	-	-	0.7
Issatchenkia orientalis FSMPY11	-	-	0.7	0.75
Kluyveromyces marxianus FSMPY39	0.6	0.8	0.9	1
Candida catenulata FSMPY25	-	-	0.7	0.7
Clavispora lusitaniae FSMPY31	-	-	0.6	0.8
**Kodamaea ohmeri* FSMPY10	0.6	0.7	0.9	1.4

The above results suggest that peptides prepared from acid casein by proteolysis with chymosin had an inhibitory effect against divers Gram-positive and Gram-negative bacteria and yeasts. This is consistent with the previous studies reporting the preparation of peptides with antimicrobial effect from casein. Lahov *et al.* (1971) prepared antimicrobial peptides, termed "casecidins", by heating and chymosin digestion of α-casein. Casecidins exerted inhibitory effects against *Staphylococcus* spp*, Bacillus subtilis*, *Diplococcus pneumonia* and *Streptococcus pyogenes.* However, "casecidins" were found to be only effective at concentrations significantly higher than those of antibiotics, which promoted the quest for other casein-derived peptides that could have antimicrobial activity, when applied at lower concentrations. Further studies identified "isracidin", "casocidin-I" and "kappacin" as other antimicrobial peptides, that were prepared from bovine

α_{s1}-casein, α_{s2}-casein, and k-casein, respectively (Hill *et al*., 1974; Zucht *et al*., 1995; Lahov and Regelson, 1996; Malkoski *et al*., 2001). The use of "isracidin" at concentrations comparable to those of antibiotics could protect mice against lethal infection by *Staphylococcus aureus* strain Smith (Lahov and Regelson, 1996). Isracidin also protected mice against *Candida albicans*, by stimulation of both phagocytosis and immune responses. Casocidin-I was shown to inhibit *Escherichia coli,* and *Staphylococcus carnosus* (Zuchet *et al*., 1995), whereas "kappacin" was inhibitory against *Streptococcus mutans* (Malkoski *et al*., 2001). More recently, McCann *et al*. (2005 & 2006) prepared and characterized new antimicrobial peptides from bovine α_{s1}- and α_{s2}-casein These peptides had inhibitory effect against Gram-positive and Gram-negative bacteria including *Listeria innocua* and *Salmonella* ser. Typhimurium, respectively. While the present work is consistent with previous studies described above, it reports for the first time the preparation of antimicrobial peptides from acid casein extracted from buffalo's milk. The ability of these buffalo's casein peptides to inhibit the variety of microorganisms examined in this study presents them as potential natural preservatives for food products. Microorganisms considered in the present work involved important food pathogens and microbial contaminants. Among those microorganisms, *Staphylococcus aureus* represents a leading cause of food poisoning due to its ability to produce enterotoxins that could provoke the so-called "toxic shock syndrome" (Ash 1997, Blaiotta *et al*. 2006). *Salmonella* ser. Typhimurium, *Shigella flexneri* and pathogenic *Escherichia coli* are important causative agents of diseases and conditions related to the gastrointestinal tract. *Cronobacter sakazakii* (formerly *Enterobacter sakazakii*) is an emerging opportunistic pathogen that causes bacteremia, necrotizing enterocolitis and meningitis, with case fatality rates as high as 80% (Bowen, 2006). *Klebsiella pneumoniae* can cause destructive changes to human lungs and inflammation (Podschun *et al*. 1998). *Issatchenkia orientalis, Kluyveromyces marxianus, Candida catenulate, Clavispora lusitaniae,* and *Kodamaea ohmeri* are yeast species that have been reported to contaminate fermented dairy products, with *Candida albicans* being a pathogenic yeast that causes candidiasis or "thrush" in humans (Fleet 2007). Based on the ability of buffalo's milk casein peptides described in this work to inhibit the above pathogenic and contaminating microorganisms, they could be recommended for use as natural preservatives of dairy products. These peptides can be prepared as outlined in the present study, and could be also generated during cheese making, where chymosin in rennet induces casein proteolysis leading to the formation of antimicrobial peptides.

REFERENCES

Ash, M. (1997). *Staphylococcus aureus* and staphylococcal enterotoxins. In *Foodborne Microorganisms of Public Health Significance.* pp 313–332. Hocking A D, Arnold G, Jenson I, Newton K, and Sutherland P, eds. North Sydney: Australian Institute of Food Science and Technology Inc.

Barentt, A.J. and G. Abdel-Tawab. (1957). Rapid method for determination of lactose in milk and cheese. Journal of the Science of Food and Agriculture 7:437.

Blaiotta, G., Fusco, V., von Eiff, C., Villani, F. and Becker, K (2006). Biotyping of enterotoxigenic *Staphylococcus aureus* by enterotoxin gene cluster (*egc*) polymorphism and spa typing analyses. *Applied and Environmental Microbiology* 72:6117–6123.

Bowen, A.B., and Braden, C.R. (2006). Invasive *Enterobacter sakazakii* disease in infants. Emerging Infectious Disease 12:1185-1189.

Darwish, S. M. (2011). Incidence of *Klebsiella ssp*. In milk and dairy products. PhD thesis, Faculty of Agriculture, Mansoura University.

El-Sharoud, M. W., and Spano, G. (2008). Diversity and enterotoxigenicity of Staphylococcus spp assoicated with Domiati cheese. Journal of Food Protection 71:2567-2571.

El-Sharoud, M. W., O'Brien, S., Negredo, C., Iversen, C., Fanning, S., and Healy, B. (2009a). Characterization of Cronobacter recovered from dried milk and related products. BMC Microbiology 9:24.

El-Sharoud, M. W., Belloch, C., Peris, D., and Querol, A. (2009b). Molecular Identification of Yeasts Associated with Traditional Egyptian Dairy Products. Journal of science 74: 341-346.

Fleet, G.H. (2007). Yeasts in foods and beverages: impact on product quality and safety. *Current Opinion in Biotechnology* 18:170–175.

Floris, R., Recio, I., Berkhout, B., and Visser, S. (2003). Antibacterial and antiviral effects of milk proteins and derivatives thereof. Current Pharmaceutical Design 9: 1257–1275.

Hill, R. D., Lahov, E., and Givol, D. (1974). A rennin-sensitive bond in alpha and beta casein. Journal of Dairy Research 41: 147-153.

Ibrahim, H. R., Aoki, T., and Pellegrini, A. (2002). Strategies for new antimicrobial proteins and peptides: Lysozyme and aprotinin as model molecules. Current Pharmaceutical Design 8: 671–693.

Kim, Y. A., and Thayer, W. D. (1996). Mechanism by which gamma irradiation increases the sensitivity of *Salmonella typhimurium* ATCC 14028 to heat. Applied and Environmental Microbiology, 62:1759-1763.

Kitts, D. D., and Weiler, K. (2003). Bioactive proteins and peptides from food sources. Applications of bioprocesses used in isolation and recovery. Current Pharmaceutical Design 16: 1309-1323.

Lahov, E. and Regelson, W. (1996). Antibacterial and Immunostimulating Casein-derived Substances from Milk: Casecidin, Isracidin Peptides. Food Chemical Toxicology 15: 131-145.

Lahov E., Edelstein, D., Sode-Mogensen, M. T. and Sofer, E. (1971). Properties of basic glycopeptides released from cow milk protein by heat. Milchwissenschaft 26: 489-495.

Lange, R., and Hengge-Aronis, R. (1991). Identification of a central regulator of stationary-phase gene expression in *Escherichia coli*. Molecular Microbiology 5:49-59.

Ling, E.R. (1963). A Text-Book of Dairy Chemistry-Practical, vol. 2. London: Chapman and Hall.

Lopez-Exposito, I., Gomez-Ruiz, J.A., Amigo, L. and Recio, I. (2006). Identification of antibacterial peptides from ovine α_{s2}-casein. International Dairy Journal 16:1072-1080.

Malkoski, M., Dashper, S. G., O'Brien-Simpson, N. M., Talbo, G. H., Macris, M., Cross, K. J., *et al*. (2001). Kappacin, a novel antibacterial peptide from bovine milk. Antimicrobial Agents in Chemotherapy 45: 2309 - 2315.

McCann, K.B., Shiell, B.J, Michalski, W.P., Lee, A., Wan, J., Roginski, H. and Coventry, M.J. (2006). Isolation and characterisation of a novel antibacterial peptide from bovine α_{S1}-casein. *International Dairy Journal* 16: 316-323.

McCann, K. B., Shiell, B. J., Michalski, W. P., Lee, A., Wan, J., Roginski, H., et al. (2005). Isolation and characterisation of antibacterial peptides derived from the f (164–207) region of bovine α_{s2}-casein. *International Dairy Journal*, 15: 133–143.

Morr, V. C. (1982). Functional properties of milk proteins and their use as food ingredients. In *Developments In Dairy Chemistry*. pp375-399. Fox F P, ed. England: Applied science publishers LTD

Nassib, T.A., Zin El-Din, M. and El-Sharoud, W.M. (2006). Effect of thermophilic lactic acid bacteria on the viability of *Salmonella* ser. Typhimurium PT8 during milk fermentation and preparation of buffalo's yoghurt. *International Journal of Dairy Technology* 59: 29-34.

Park, C. B., Lee, H. L., Park, I. Y., Kim, M. S., and Kim, S. C. (1997). A novel antimicrobial peptide from the loach, Misgurnus Anguillicaudatus. FEBS Letters 411: 173–178.

Podschun, R., and Ullman, U. (1998). "*Klebsiella* spp. as Nosocomial Pathogens: Epidemiology, Taxonomy, Typing Methods, and Pathogenicity Factors". *Clinical Microbiology Reviews* 11: 589–603.

Reinheimer, J. A., Demkow, M. R. and Condioti, M. C. (1990). Inhibition of coliform bacteria by lactic cultures. Australian Journal of Dairy Technology 45: 5-9.

Segura, A., Moreno, M., Molina, A., and Garcia-Olmedo, F. (1998). Novel defensin subfamily from spinach (Spinacia oleracea). FEBS Letters 435: 159–162.

Shimizu, M. (2004). Food-derived peptides and intestinal functions. *BioFactors* 21: 43–47.

Silhavy, T. J., Berman, L. M., and Enquist. W. L. (1984). Experiments with gene fusions, p. 107–112. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Singh, J. and Laxminarayana, H. (1973). Indian Journal of Dairy Science. Antibacterial activity of lactobacilli 26: 135-136.

Wakabayashi, H., Takase, M., and Tomita, M. (2003). Lactoferricin derived from milk protein lactoferrin. Current Pharmaceutical Design 16: 1277 - 1287.

Zucht, H. D., Raida, M., Adermann, K., Magert, H. J., and Forssman, W. G. (1995). Casocidin-I: A casein αs2-derived peptide exhibits antibacterial activity. FEBS Letters 372:185–188.

دراسة الفاعلية المضادة للميكروبات للببتيدات الناتجة عن تحلل كازين اللبن الجاموسي

الطاهرة أحمد محمد عمار و عبد الوهاب الشاذلي خليل و وليد محمود الشارود و سمر أحمد زلمة

قسم الألبان- كلية الزراعة- جامعة المنصورة.

أستهدفت هذه الدراسة تحضير ببتيدات من كازين اللبن الجاموسي ودراسة فاعلية هذه الببتيدات ضد مجموعة متنوعة من الميكروبات الملوثة للأغذية. حيث تم تحضير الكازين بطريقة الترسيب الحامضي من اللبن الفرز الجاموسي ووُجد أن الكازين الناتج يتكون من البروتين (٨٣,١٩%)، والماء (١١,٤٥%)، والرماد (٢,٦%)، والدهن (١,٦%)، واللاكتوز (٠,٥٣%). ثم تم تحضير ببتيدات بالتحلل الانزيمي للكازين الحامضي الناتج بإستخدام إنزيم الكيموسين، مع تحضير محاليل تحتوي علي تركيزات ١٠%، ٢٠%، ٣٠%، ٤٠% (وزن/حجم%) من هذه الببتيدات. وتم إختبار فاعلية هذه التركيزات ضد نمو مجموعة من البكتريا الموجبة والسالبة لجرام والخمائر. وقد وُجد أن الفاعلية ضد الميكروبات اختلفت بإختلاف نوعية الميكروب المختبر وتركيز محلول ببتيدات الكازين. حيث أمكن تثبيط بكتريا *السالمونيلا* طراز سيريولوجي تيفيميوريم، وبكتريا *الكرونوبكتر ساكازاكي*، وخميرة *الكلوفيروميسس ماركسينس*، وخميرة *كوداميا أومري* بدرجات متفاوتة بواسطة جميع تركيزات ببتيدات الكازين المستخدمة والتي تراوحت من ١٠% إلي ٤٠%. بينما لم يمكن تثبيط بكتريا *الايشريشيا كولاي* و*الكلبسيلا نيومونيا* إلا بتركيزات أعلي من ١٠%. كذلك فإنه لم يمكن تثبيط بكتريا *الشيجلا فليكسنري*، وخميرة *إستاتشنيكا أورينتالس*، وخميرة *الكانديدا كاتنيولات*، وخميرة *الكلافيسبورا لوسيتانيا* إلا بتركيزات أعلي من ٢٠%. وقد أظهرت خميرة *الكانديدا ألبيكانس* أعلي مقاومة ممكنة لتأثير ببتيدات الكازين حيث لم يكن من الممكن تثبيطها إلا بإستخدام تركيزات تصل إلي ٤٠%. وبالتالي فإن هذه النتائج تدل علي أن الببتيدات الناتجة من تحلل كازين اللبن الجاموسي بواسطة إنزيم الكيموسين لها فاعلية مضادة لنشاط عدد متنوع من البكتريا الموجبة والسالبة لجرام، والخمائر.

قام بتحكيم البحث

أ.د / طه عبد الحليم نصيب	**كلية الزراعة – جامعة المنصورة**
أ.د / نبيل محمد مهنا	**كلية الزراعة – جامعة كفر الشيخ**