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Natural Antimicrobial Compounds as Meat Preservatives

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ABSTRACT

Current trends in consumer interests indicate an increasing demand for the use of natural preservatives in foods. Meat products, which are highly perishable and carry risks of food-borne pathogens, have been preserved in a variety of ways, including via the use of organic acids, nitrites/nitrates, and modified atmosphere packaging. Certain natural antimicrobials have been shown to be effective at inhibiting a wide range of microbes. Researches in this area have recently concentrated on the application of these natural antimicrobials to meats as a way to preserve these perishable foods against spoilage and pathogenic microbes. This review presents the most well-studied natural antimicrobial agents that have been applied to meat products, including bacteriocins, chitosan, lactoferrin, lactoperoxidase system, monolaurin and bacteriophages.

Keywords: bacteriocin, bacteriophage, chitosan, lactoferrin, lactoperoxidase system, monolaurin

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INTRODUCTION

Various food preservation systems have been developed to reduce the incidence of microbial food spoilage and food poisoning related to contaminated foods. These include heat treatment, drying, modified atmosphere packaging, chemical preservatives and irradiation (Gould 1995; Rahman 1999). However, some of these systems could be energy-consuming, entail the use of undesirable additives, or require specially trained personnel to conduct. The use of *natural* antimicrobial compounds in foods as an alternative is driven not just by the effectiveness of the compounds but by consumer demands for fresher, more natural tasting and less synthetic chemicals that are added to foods (Dillon *et al*. 1994). Since the mid 1980s, research in this area has grown tremendously, resulting in the approval by the United States Food and Drug Administration (FDA) of a number of natural antimicrobials for use as food additives within the last two decades. Much work has concentrated on the studies of natural antimicrobials against spoilage and pathogenic organisms *in vitro*, and although success has occurred with the application of these products in test tubes, their effectiveness is typically less pronounced when applied to a complex food matrix. Meat products, in particular, are high in protein, fat and moisture, factors that help protect microorganisms and neutralize the effects of antimicrobials. However, some compounds do exhibit significant antimicrobial properties in foods and this review will cover the most well studied natural compounds that have been and are currently applied to meat products.

BACTERIOCINS

Bacteriocins are one of the most well studied natural antimicrobial systems that have been applied in food products. Bacteriocins are ribosomally synthesized bacterial proteins or peptides that can kill or inhibit other bacteria. There are various kinds of bacteriocins, mostly from lactic acid bacteria. These bacteriocins are classified in three main groups: (1) the modified bacteriocins or lantibiotics (Class I) which undergo extensive posttranslational modification to produce an active peptide and are featured by their dehydrate residues (dehydroalanine, dehydrobutyrine), lanthionine, and beta-methyl lanthionine, with molecular weights (MW) of 2-5 kDa (Adams *et al*. 2003); (2) the heat-stable unmodified bacteriocins (Class II) with MW of less than 10 kDa; and (3) the larger heat-labile bacteriocins (Class III) with MW of more than 30 kDa (Papagianni 2003). Class IV bacterio-

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cins have been specified as complex molecules consisting of a protein moiety and one or more other chemical moieties (Papagianni 2003). Recently, a new class, Class V bacteriocins, has been demonstrated as "cyclic bacteriocins", featured by the structure that their N- and C-terminals are covalently linked together (Kawai *et al.* 2004).

Bacteriocins are applicable to meat products in several ways, including the use of bacteriocin-producing starter cultures, in active packaging, in meat batter, and sprayed on meat surfaces (Hugas *et al.* 2002). The bacteriocins that have been applied to meat products mainly belong to Class I (lantibiotics), Class IIa (pediocin-like, cystibiotic, or antilisterial type peptides), some Class IIb, and Class V bacteriocins. In general, non-lantibiotics have a narrower spectrum of inhibition than the lantibiotics (Papagianni 2003). Bacteriocins that are effective against food pathogenic and spoilage bacteria and applied in animal products, have previously been reviewed (Adams *et al.* 2003; Dawson *et al.*

Table 1 Bacteriocins and their application in meat and meat products.

Bacteriocin	GRAS[1]	Meat application	Reference
Nisin	Yes	Beef muscle slices and ground beef	Millette *et al.* 2007
		Beef surface	Cutter *et al.* 1995a, 1995b, 1996
		Chicken carcass	Gogus *et al.* 2004
		Cooked ham	Jofre *et al.* 2007
		Cooked meat products (bologna, frankfurter)	Danisco 2002
		Fat and lean pork tissue	Nattress *et al.* 2001, 2003
		Mechanically recovered portly meat	Yuste *et al.* 2000
		Pork loins	Nattress *et al.* 2003
		Surface of full-fat turkey frankfurters	Sivarooban *et al.* 2007
		Turkish fermented sausages	Hampikyan *et al.* 2007
		Vacuum packaged fresh beef	Mustapha *et al.* 2002
Pediocin	No	Semidry sausage	Berry *et al.* 1990
		Fresh meat surface	Nielsen *et al.* 1990
		Ground beef, sausage mix	Motlagh *et al.* 1992
		Fermented sausage	Foegeding *et al.* 1992; Marilao *et al.* 2007
Leucocin	No	Dry fermented sausage[2]	Mataragas *et al.* 2002
		Vacuum-packaged sucuk[2]	Osmanagaoglu 2007
Enterocin	No	Sliced cooked ham	Marcos *et al.* 2007; Jofre *et al.* 2007
Sakacin	No	Vacuum-packaged beef slices	Katikou *et al.* 2005
		Cooked ham/ sliced cooked ham	Vermeiren *et al.* 2006
		Fermented sausage	Hugas *et al.* 1995; Drosinos *et al.* 2006; Urso *et al.* 2006
		Sliced cooked ham	Hequet *et al.* 2007
		Raw minced pork, poultry breasts, cooked pork	Hugas *et al.* 1998
Curvaticin	No	Pork	Ghalfi *et al.* 2006
Piscicocin	No	Ground meat	Azuma *et al.* 2007
Lactocin	No	Ground beef	Vignolo *et al.* 1996
		Dry fermented sausage[2]	Palacios *et al.* 1999
Acidocin	No	Cooked pork surface/ raw ground pork	El-Ziney *et al.* 1999
Pentocin	No	Xuan-Wei Ham[2]	Liu *et al.* 2008

[1]Generally Recognized as Safe
[2] Indicates the original source of the bacteriocin-producing strain.

Table 2 Natural antimicrobials and their application in meat and meat products.

Compound	GRAS[1]		Meat application	Reference
Chitosan	Yes		Beef patties, fermented meat products	Darmadji *et al.* 1994
			Raw minced pork	Sagoo *et al.* 2002
			Skinless and standard raw pork sausage	Sagoo *et al.* 2002
			Cooked ground beef and turkey	Juneja *et al.* 2006
			Cured Korean sausage	Youn *et al.* 1999
			Spicy beef	Youn *et al.* 2004
			Grilled pork	Yingyuad *et al.* 2006
			Chilled fresh pork sausage	Roller *et al.* 2002; Georgantelis *et al.* 2007
			Fresh mutton kababs, streaky bacon	Rao *et al.* 2005
			Bologna	Zivanovic *et al.* 2005
			Minced lamb meat, pork cocktail salami	Kanatt *et al.* 2008a, 2008b
			Bologna, pastrami, cooked ham	Ouattara *et al.* 2000
Lactoferrin	Yes		Beef steak	Naidu 2002
			Bologna	Al-Nabulsi 2006b
			Hot-boned ground pork	Chiu *et al.* 2007
			Dry cured sausage	Al-Nabulsi *et al.* 2007
Lacto-peroxidase	Yes		Poultry	Wolfson *et al.* 1994
			Red and ground meat	Kennedy *et al.* 2000
			Beef cubes	Elliot *et al.* 2004
			Marinated broiler drumsticks	Tan *et al.* 2006
			Ground beef	McLay *et al.* 2002
Monolaurin	Food emulsifier		Meat products	Kabara 1984
			Beef emulsions, hot dogs	Mbandi *et al.* 2004
			Ground beef	McLay *et al.* 2002
Bacteriophage	LISTEX™ P100	Yes	All food, against *L. monocytogenes*	EBI Food Safety 2005
	LMP-102™	Food additive	RTE meat and poultry, against *L. monocytogenes*	Intralytix Inc. 2006
	ECP-100™	No	Against *E. coli* O157:H7	Intralytix Inc. 2007

[1]Generally Recognized as Safe

2003; Ray *et al.* 2003; Tyopponen *et al.* 2003). Generally, bacteriocins are much more stable in cooked meat products than in raw products. This is because bacteriocins are amphiphilic peptides susceptible to binding to food macromolecules and proteolytic degradation, resulting in their inactivation (Murray *et al.* 1997; Aasen *et al.* 2003). According to Aasen *et al.* (2003), more than 80% of added sakacin P and nisin were quickly adsorbed to proteins in meat matrices, including homogenates of cold-smoked salmon, cooked chicken, cold cuts and raw chicken. Less than 1% of the total bacteriocins remained in raw chicken after 1 week of storage at 10°C (Aasen *et al.* 2003). Bacteriocins are also more effective when applied to meat surfaces than to homogenized meat or meat products, such as liquid food and forcemeat (Aasen *et al.* 2003). It may be that fats present in liquid food and forcemeat may inactivate the bacteriocins more significantly than on intact meat surfaces (Aasen *et al.* 2003). A list of bacteriocins that have been studied in meat and meat products are shown in **Table 1**. Other natural compounds that have been studied in meats are listed in **Table 2**.

Class I Bacteriocins

Class I bacteriocins are further classified into six subgroups (Papagianni 2003). Currently, lantibiotics that have been studied in meat products are mainly nisin A and Z.

Nisin

Nisin is a 34-amino-acid peptide which has a pentacyclic structure: one lanthionine residue (ring A) and four beta-methyllanthionine residues (rings B, C, D and E) (Shiba *et al.* 1991). It is produced by certain strains of *Lactococcus lactis* subsp. *lactis*. Nisin is first synthesized as a precursor peptide in the bacterial cell, modified post-translationally to form the mature nisin, and finally exported from the cell. Nisin is classified as a Class Ia bacteriocin or lantibiotic (Klaenhammer 1993). Generally, there are two natural variants of nisin, nisin A and nisin Z, the difference of which lies in the amino acid at position 27 with asparagine in nisin A and histidine in nisin Z (Mulders *et al.* 1991). In the United States, nisin is considered as a Generally Recognized as Safe (GRAS) substance by the FDA.

Mechanisms of antimicrobial activity

Nisin is effective at inhibiting gram-positive bacteria and their spores. However, it has no antimicrobial activity against gram-negative bacteria, yeasts, or molds (Delves-Broughton *et al.* 1996). The mechanism of antimicrobial activity of nisin against Gram positive bacteria is found to depend on two functions. First, nisin binds to lipase II, which is a membrane-bound precursor protein involved in cell-wall biosynthesis. Then, it diminishes the proton motive force of the cell by forming a pore through the cytoplasmic membrane, which results in leakage of essential cell constituents, including ATP, amino acids and various ions (Breukink *et al.* 1999; Wiedemann *et al.* 2002). The biochemical basis of nisin activity against spores is still not well understood, but it is normally sporistatic (Adams *et al.* 2003). On the other hand, the resistance of Gram negative bacteria to nisin inhibition is attributed to their protective outer membrane that excludes the free passage of the comparatively high MW nisin (Adams *et al.* 2003). Even though nisin is heat stable (Hurst 1981), it may only have significant antimicrobial activity during the early (24-h) period of refrigerated storage (Rose *et al.* 1999). Other factors that may affect its antimicrobial effects include its solubility (insoluble in non-polar solvents), and pH values of the medium. The optimal pH of nisin activity is ca. 3.0 when testing against *Listeria monocytogenes* (Davies *et al.* 1994).

Application in meat products

Nisin alone

According to Danisco (2002), the commercialized nisin, Nisaplin®, has been used at a level of 200-400 mg per kg/L in cooked meat products, including bologna and frankfurter sausages, mainly to inhibit lactic acid bacteria, *Brochothrix thermosphacta* and *L. monocytogenes*. However, little success has been reported on the use of nisin in raw meat products. Analyses of pure nisin in meats suggest that nisin reacts enzymatically with glutathione and is subsequently inactivated (Rose *et al.* 1999). Also, the enzyme leading to this reaction – glutathione *S*-transferase – has been shown by isolation to be plentiful in fresh meat (Rose *et al.* 2002). In addition, nisin binding to meat particles and fat may also contribute to its loss of activity (Aasen *et al.* 2003).

A study conducted by Hampikyan *et al.* (2007) demonstrated that 50 and 100 μg/g nisin could significantly ($P \leq 0.001$) eliminate *L. monocytogenes* in Turkish fermented sausages (sucuks) at day 25 and day 20, indicating that the inhibition of this pathogen in sucuk increases with increasing concentrations of nisin. Nisin incorporated into calcium alginate gel has been demonstrated to be more effectively delivered to carcass surfaces than free nisin. According to Cutter *et al.* (1996), 100 μg/ml nisin immobilized in a calcium alginate gel could suppress *B. thermosphacta* on beef surface from approximately 4.5 $\log_{10}$ cfu/cm^2 to greater than 2.42 $\log_{10}$ cfu/cm^2 at the end of seven days, compared with no suppression of the spoilage bacterium by 100 μg/ml unimmobilized nisin. Also, Millette *et al.* (2007) demonstrated that by incorporating nisin into hydrophobic and biodegradable films or beads, it was much more efficient to control the growth of pathogens or spoilage microorganisms on the surface of round beef or other meat products.

Bacteriocin-producing strains may be used directly in food. For example, bacteriocin-producing lactic acid bacteria have been used in traditional fermented meat products, such as salami, which highlights another way of using bacteriocins, since these lactic acid bacteria can produce bacteriocin *in situ*.

Nisin combined with other hurdles

Research on nisin hurdles has been conducted to widen the application spectrum of this compound by combination with other antimicrobials. Nisin combined with lactic acid showed enhanced antimicrobial effects to various Gram negative bacteria. According to Gogus *et al.* (2004), a 5-h yogurt dip before nisin treatment with or without an oil-wax coating of chicken carcass demonstrated significant inhibition of mesophilic aerobic bacteria and *Salmonella* of 2.11 and 1.97 log reductions, respectively. However, these acids could not enhance the effect of nisin against the Gram negative pathogen, *Escherichia coli* O157:H7, for vacuum packaged beef (Mustapha *et al.* 2002).

Nisin mixed with grape seed extract also showed significant inhibition of growth of *L. monocytogenes* on ready-to-eat (RTE) meat, despite a high fat content. Sivarooban *et al.* (2007), reported that the combination of nisin (6,400 IU/ml) and grape seed extract (1%) resulted in the greatest inhibitory activity on the surface of full-fat turkey frankfurters with reductions of *L. monocytogenes* populations to undetectable levels after 21 days.

In nonpressurized cooked ham, the active packaging with the nisin and lactate-containing interleavers were strongly effective at inhibiting *L. monocytogenes* growth for 30 days at 6°C (Jofre *et al.* 2007).

Nisin combined with lysozyme showed synergistic effects against gram-positive meat-related pathogens, including *L. monocytogenes*, and meat-related spoilage lactobacilli (Monticello 1989; Chung *et al.* 2000). Nattress *et al.* (2001) demonstrated that on fat and lean pork tissues, mixtures of nisin and lysozyme were more effective than each alone. Specifically, a 1:3 combination of nisin/lysozyme at a surface concentration of 260 μg/cm^2 was more effective than either single agent at reducing the numbers of *Carnobacterium* sp. at 0 and 3 days in pork juice, and at 0 day on lean pork tissue. This combination was also effective at controlling the growth of lactic acid bacteria, including those that are able to grow in the presence of acetate, and *B.*

thermosphacta, on naturally contaminated pork loins that were stored in vacuum packages at 2°C for up to 6 weeks (Nattress *et al.* 2003). It is speculated that lysozyme may facilitate the access of nisin to the target cell membrane, or it could be that nisin inhibits the repair of lysozyme damage by the cells (Adams *et al.* 2003).

Nisin combined with high pressure processing (HPP) showed a promising antimicrobial effect against aerobic mesophilic and psychrotrophic bacterial populations of mechanically recovered portly meat (Yuste *et al.* 2000).

Nisin combined with chelators are known to confer synergistic effects against Gram negative bacteria (Gill *et al.* 2003). For example, nisin combined with EDTA, sodium phosphate tripoly or citrate has been shown to inhibit *Salmonella*, *E. coli* O157:H7 and *Shigella* in buffer (Stevens *et al.* 1991, 1992a, 1992b; Cutter *et al.* 1995a). The inhibition mode is that the chelator adsorbs the divalent cations, Ca^{2+} and Mg^{2+} in the buffer, which are essential for the stability of the gram-negative outer membrane, by acting as salt bridges between membrane macromolecules, such as lipopolysaccharides, thus disrupting it. This, in turn, acts to assist nisin in pore forming. However, when this model is constructed in a nutrient medium (nutrient broth or meat products), such a combination could not inhibit Gram negative bacteria. According to Cutter *et al.* (1995b), nisin/EDTA cannot significantly inhibit *E. coli* and *Salmonella* on beef. Further, no increase in antimicrobial activity has been observed on *E. coli* O157:H7 and *Salmonella* Typhimurium in nutrient broth (Gill *et al.* 2003). Also, Mustapha *et al.* (2002) reported that such a combination did not show significant effects against *S.* Typhimurium on vacuum-packaged fresh beef. These previous studies suggest that in media capable of supporting growth, chelators may not offer much synergistic effects for nisin inhibition of Gram negative bacteria (Gill *et al.* 2003).

A combination of multiple inhibitors with nisin has been shown to exhibit synergistic effects. Nisin combined with lysozyme and EDTA showed inhibition of growth of certain meat-related gram-negative bacteria. Gill *et al.* (2000) reported that the addition of 500 mg/kg lysozyme + nisin, at a ratio of 1:3, and 500 mg/kg EDTA to ham prior to cooking reduced the growth of *E. coli* O157:H7 for 4 weeks at 8°C, and exhibited inhibitory effect on *S.* Typhimurium after 3 weeks at 8°C. High hydrostatic pressure combined with nisin and lysozyme, demonstrated a promising way to avoid post-processing contamination of meat products, because such a combination could cause lethal injuries to bacterial cell membranes, thus increasing the death rate of living cells (Garriga *et al.* 2002).

Class II bacteriocins

Class IIa bacteriocins

Class IIa bacteriocins, also called pediocin-like bacteriocins, cystibiotics, or anti-listerial type peptides, are generally small unmodified peptides with MWs less than 5 kDa and are characterized by a –Y-G-N-G-V N-terminus. These are known for their inhibitory effects against *L. monocytogenes*.

Class IIa bacteriocins that have been isolated from meat products include pediocin PA-1/AcH, enterocin A and P13, leucocin A, sakacin A, P and 674, curvacin A, bavaricin MN, carnobacteriocin B2, and piscicocin 126 (Ray *et al.* 2003). Currently, pediocin-like bacteriocins that have been studied in meat products are mostly pediocin, leucocin and enterocin. The antimicrobial activity of a pediocin-like bacteriocin is initiated by its binding to bacterial cells with the assistance of the N-terminal region (Chikindas *et al.* 1993; Fimland *et al.* 1996), which leads to formation of ion conductance pores in the cell membrane. With prolonged exposure to at least some cystibiotics, autolytic enzymes within the cell wall are activated, leading to cell lysis (Ray *et al.* 2003).

Pediocin

Pediocins are mostly produced by some strains of *Pediococcus* spp., including *Pediococcus acidilactici*, *Pediococcus pentosaceus*, and *Pediococcus parvulus*. Among them, pediocin PA-1/AcH was the first and most thoroughly studied pediocin. It is reported that pediocin PA-1/AcH is produced by *P. acidilactici* (Nieto Lozano *et al.* 1992; Cintas *et al.* 1995), *P. parvulus* (Bennik *et al.* 1997) and *Lactobacillus plantarum* (Loesner *et al.* 2003).

Pediocin PA-1 produced by *P. acidilactici* was stable and had an inhibitory and bactericidal effect on *L. monocytogenes* for up to 28 days of refrigerated storage on meat surfaces (Nielsen *et al.* 1990). Pediocin AcH produced by *P. acidilactici* H was also shown to have a bactericidal effect against *Listeria ivanovii* and *L. monocytogenes* inoculated on meat (Motlagh *et al.* 1992). Further, pediocin produced by *P. pentosaceus* has been identified from "Alheiras", traditional Portuguese fermented sausages, to have antagonistic activity against *Listeria innocua* and *L. monocytogenes* (Albano *et al.* 2007). In addition, the pediocin-producing strain, *P. acidilactici* AA5a, was shown to have effective inhibition against the growth of putative *Listeria* within 12-20 h of pork sausage fermentation, although no significant antilisterial effect was found after the fermentation (Marilao *et al.* 2007). In addition to *Listeria* spp., pediocins PA-1/AcH are also indicated to have inhibitory or bacteriostatic effects against other meat-related pathogenic and spoilage bacteria, including *Clostridium perfringens*, *Clostridium laramie* and *Leuconostoc* spp. (Ray *et al.* 1989; Kalchayanand *et al.* 1992; Nieto-Lozano *et al.* 2006).

The antilisterial activity of pediocin lies in the existence of two cystine bridges in the molecule, which has been reported to be more active and with a broader inhibitory spectrum than those with only one cystine bridge (Loesner *et al.* 2003). Further, monomers of pediocin are more stable and effective than oligomers of the molecule. The antilisterial activity of pediocin depends on the pH of the medium and protein concentration of the matrix. Specifically, high pH (6-8) and high protein concentration (more than 0.55 mg/ml) could induce the formation of dimers, so that the antilisterial activity of pediocin is decreased (Abriouel *et al.* 2001). According to Berry *et al.* (1990), inhibition of *L. monocytogenes* has been detected by bacteriocin-producing *Pediococcus* spp. in semidry sausage. However, since the pH of the product was higher than 5.5, this bacteriocin could not eliminate surviving *L. monocytogenes* after heating to an internal temperature of 64.4°C and storage for 2 weeks. In contrast, according to Foegeding *et al.* (1992), pediocin produced by *P. acidilactici* PAC 1.0 *in situ* showed effective inactivation of *L. monocytogenes* when the pH was <4.9 at the end of fermentation and also enhanced the inhibition of *L. monocytogenes* during drying in the process of fermented sausage manufacturing.

The MW of pediocin is quite small. A pediocin produced by *P. parvulus* strain 133, isolated from a traditional Mexican meat product, chorizo, was estimated to be 5 kDa (Schneider *et al.* 2006). The isoelectric point of the molecule has also been studied, with the one from *P. parvulus* 133 being 8.65 (Schneider *et al.* 2006) and pediocin PA-1/AcH at 9.6 (Ray *et al.* 2000). This bacteriocin also has high thermostability. According to Schneider *et al.* (2006), the pediocin from *P. parvulus* 133 remained active after heating to 121°C for 15 min. However, its thermostability depends on the pH. The higher the pH, the more sensitive it is to heat. It was reported that after heating to 121°C for 15 min at pH 4, 6, 7 and 8, 11%, 84%, 100% and 100%, respectively, of the pediocin activity was lost.

Leucocin

Leucocins are small heat-stable peptides with MWs of 3.5-4.0 kDa. This molecule is produced by several bacteriocin-producing *Leuconostoc* strains isolated from meat, including *Leuconostoc gelidum* UAL 187, *Leuconostoc paramesenteroides*-La7a, *Leuconostoc carnosum*-Ta11a and *L. carnosum*-La54a (Hastings *et al.* 1994). According to Mataragas *et al.* (2002), leucocin produced by *Leuconostoc mesenteroides* L124, isolated from dry fermented sausage, re-

mained active even after heating at 100°C for 60 min. Osmanagaoglu (2007) reported that leucocin OZ produced by *L. carnosum* isolated from vacuum-packaged sucuk, a dry and spicy sausage eaten from the Balkans to the Middle East, was resistant to heating at 121°C for 15 min. Leucocins are stable at a large pH spectrum, while their antimicrobial activity is greater at low pH. Leucocin A-UAL187, produced by *L. gelidum* UAL 187, has been shown to be active at a pH range of 4.0-7.0 (Hastings *et al.* 1994), while that produced by *L. mesenteroides* L124 preferred a pH range of 2.0-8.0 (Mataragas *et al.* 2002), and leucocin from *L. carnosum* has a pH optimum of 2-12 (Osmanagaoglu 2007). However, this bacteriocin is susceptible to proteolytic enzymes and lipase but resistant to catalase and lysozyme. The antimicrobial activity of leucocin has been demonstrated mostly against *L. monocytogenes* and other lactic acid bacteria involved in meat spoilage. It is suggested that *Leuconostoc* spp. involved in meat spoilage may be an important source of leucocin production in meat products. However, no research has been done to evaluate leucocin activity against spoilage or pathogenic microbes in meat products.

Enterocin

Among the enterocins that have been characterized so far, the majority have been ranked under Class IIa (Eijsink *et al.* 2002). Enterocins A and B are those that have been best characterized and applied in the study of meat and meat product preservation. Enterocin A, MW 4.8, is produced by *Enterococcus faecium* CTC492 and *E. faecium* T136 (both isolated from Spanish fermented sausages enterocins), and *E. faecium* BFE900 (isolated from black olives). Enterocin B, MW 5.4 kDa, is produced by *E. faecium* T136 (isolated from Spanish fermented sausages) (Aymerich *et al.* 1996; Casaus *et al.* 1997; Franz *et al.* 1999). These compounds have been proven to be effective at controlling the growth of *L. monocytogenes* in meat products (Aymerich *et al.* 2000; Vignolo *et al.* 2000; Ananou *et al.* 2005). Enterocin B does not belong to Class IIa bacteriocins, but is similar and related to Class IIa bacteriocins with respect to its chemical characteristics, heat stability, and antilisterial activity (Casaus *et al.* 1997).

Active packaging containing enterocins has been shown to improve the safety of sliced cooked ham by delaying and reducing the growth of *L. monocytogenes* (Marcos *et al.* 2007). In addition, HPP could improve the antilisterial effect of the enterocin-containing active package, resulting in a decrease of *Listeria* populations by about 4 log cfu/g during storage of the packaged ham slices (Jofre *et al.* 2007).

Sakacin

De Martinis *et al.* (1998) suggested that a bacteriocin-like substance produced by *Lactobacillus sakei* 2a could be used to inhibit the growth of *L. monocytogenes* in pork. Another bacteriocin-like inhibitory substances producer, *L. sakei* CECT 4808, was also proven to have inhibitory effects against spoilage bacteria in vacuum-packaged beef slices (Katikou *et al.* 2005). *L. sakei* 10A at 10^6 cfu/g was able to limit *L. monocytogenes* to <1 $\log_{10}$ cfu/g in a model cooked ham at the end of 27 days (Vermeiren *et al.* 2006). Further, the starter culture *L. sakei* CTC494 from naturally fermented sausage was shown to be able to produce the bacteriocin sakacin K, thus not only suppressing the growth of *Listeria* (initially spiked at 9×10^3 cfu/g) but also reducing their number by 1.25 logs compared to a non-bacteriocinogenic control strain (Hugas *et al.* 1995). According to Urso *et al.* (2006), a sakacin P producing *L. sakei* strain inoculated in fermented sausages showed rapid colonization in the sausage ecosystem, and resulted in a rapid decrease of the total bacterial count and fecal enterococci at the end of the fermentation. Further, *L. sakei* 2512, demonstrated to inhibit the growth of *Listeria* on sliced cooked ham, was identified to produce sakacin G (Hequet *et al.* 2007). The addition of *L. sakei* was also shown to significantly decrease the concentration of *L. monocytogenes* in RTE fermented sausages upon consumption (Drosinos *et al.* 2006). Combined with vacuum packaging or modified atmosphere packaging, sakacin K produced by *L. sakei* CTC494 showed immediate bactericidal action against *Listeria* in fresh and cooked meat products (Hugas *et al.* 1998). Similarly, the combination of *L. sakei* 10A and 4°C or a modified atmosphere packaging containing 50% CO_2 completely inhibited the growth of *L. monocytogenes* in cooked ham (Vermeiren *et al.* 2006).

Other class IIa bacteriocins

Ghalfi *et al.* (2006) found that the bacteriocin-producing *Lactobacillus curvatus* CWBI-B28 was efficient at controlling *L. monocytogenes* in pork meat within one or two weeks with or without the presence of nitrites, but the efficacy was rapidly reduced upon extended storage at 4°C. Curvaticin, the cell-adsorbed bacteriocin produced by *L. curvatus* CWBI-B28, was combined with oregano and savory essential oil, and the combination exhibited a synergistic effect at controlling *L. monocytogenes* in pork meat during storage at 4°C (Ghalfi *et al.* 2007).

Piscicocin CS526 produced by *Carnobacterium piscicola* CS526 has been shown to be active against *Enterococcus*, *Listeria*, *Pediococcus*, and *Leuconostoc* (Yamazaki *et al.* 2005). It was inactivated by proteolytic enzymes, was stable at 100°C for 30 min, and had a pH range of 2 to 8. Its N-terminal sequence was YGNGL, but not the YGNGV N-terminus commonly found in class IIa bacteriocins. The MW of piscicocin CS526 was approximately 4.4 kDa (Yamazaki *et al.* 2005). It has been shown that a piscicocin CS526 Bac(+) fermentate was effective for the control of *L. monocytogenes* in ground meat at refrigeration temperatures (Azuma *et al.* 2007).

Other class II bacteriocins

Lactocin 705 is produced by *Lactobacillus casei* CRL 705, which is isolated from a dry fermented sausage. This bacteriocin has a MW of about 3357.80 Da, with an isoelectric point of 10.03 (Palacios *et al.* 1999). This lantibiotic has been shown to have inhibitory effects against *L. monocytogenes* in ground beef (Vignolo *et al.* 1996). *L. curvatus* CRL705 and the produced bacteriocins, lactocin 705 and lactocin AL 705, have also been shown to be effective at inhibiting *L. innocua* and *B. thermosphacta* in refrigerated vacuum-packaged fresh meat (Castellano *et al.* 2006).

According to Deraz *et al.* (2005), acidocin D20079 could be produced by *Lactobacillus acidophilus* DSM 20079. It is a small bacteriocin with a MW of 6.6 kDa and is extremely heat-stable (30 min at 121°C). However, it is sensitive to proteolytic enzymes and has a narrow inhibitory spectrum which is restricted to the genus *Lactobacillus*, including *L. sakei*, a main cause of anaerobic spoilage of vacuum-packaged meat products.

Reuterin is a bacteriocin formed during the anaerobic growth of *Lactobacillus reuteri* in the presence of glycerol (Axelsson *et al.* 1989). This bacteriocin is soluble in water and adapted to a wide range of pH. Moreover, it is resistant to proteolytic and lipolytic enzymes, and has potential activity not only against gram-positive, but also Gram negative bacteria. These advantages make reuterin a well adapted biopreservative in meat products (El-Ziney *et al.* 1999). The antimicrobial activity of reuterin produced by *L. reuteri* 120002 has been studied against *E. coli* O157:H7 and *L. monocytogenes* on cooked pork surfaces and in raw ground pork (El-Ziney *et al.* 1999). Following inoculation of *E. coli* O157:H7 and *L. monocytogenes* on cooked pork surfaces and subsequent incubation for 30 min at 7°C, the exposure to 500 AU/ml reuterin for 15 s and 24 h led to a 0.45 and 2.7 log cfu/cm^2 reduction, respectively, for *E. coli* O157:H7, and 0.3 and 0.63 cfu/cm^2, respectively, for *L. monocytogenes*. In raw ground pork, 100 AU/g resulted in a 5 log cfu/cm^2 reduction of *E. coli* O157:H7, and 250 AU/g resulted in a 3 log cfu/cm^2 reduction of *L. monocytogenes* after 1 week of storage at 7°C.

Pentocin 31-1 is a newly found bacteriocin produced by *Lactobacillus pentosus* 31-1 that is isolated from Xuan-Wei Ham, a traditional Chinese fermented meat product (Liu *et al.* 2008). It has a MW of approximately 14.2 kDa. According to Liu *et al.* (2008), this bacteriocin showed a wide range of antimicrobial activity against *Listeria* spp., *Staphylococcus* spp., *Bacillus* spp., *Lactobacillus* spp., *Streptococcus* spp., *Pediococcus* spp. and even *E. coli*. All the *Listeria* spp. tested including *L. monocytogenes*, were highly sensitive to this bacteriocin. Pentocin 31-1 was shown to be pH resistant, heat stable, and protease sensitive. Its application in meat products is promising although no research in meat products has been conducted at this point.

Class V bacteriocins

Among the bacteriocins in this group, gassericins have been shown to have antimicrobial effects against meat spoilage and pathogenic bacteria. Gassericin A, produced by *Lactobacillus gasseri* LA39 and composed of 58 amino acids residues upon maturation, has showed antibacterial activity against Gram positive foodborne pathogenic bacteria, including *L. monocytogenes*, *Bacillus cereus* and *Staphylococcus aureus*, but not Gram negative bacteria (Kawai *et al.* 2004). Zhu *et al.* (2000) reported the isolation and characterization of gassericin KT7, produced by *L. gasseri* KT7 isolated from infant feces. They found that gassericin KT7 has a MW of 4.5-5.0 kDa and is active over a wide range of pH. It showed inhibitory effect against food spoilage and pathogenic bacteria, including *Enterococcus*, *Clostridium* and *Listeria*. Gassericin is bactericidal to sensitive cells, but not by cell lysis.

CHITOSAN

Chitosan is a biodegradable, non-toxic polycationic polymer (poly-β-1,4,-linked glucosamine) commercially made by alkaline deacetylation of chitin (-1,4-N-acetyl-D-glucosamine). Chitin is mainly derived from constituents of crustacean shells and fungi. It is the second most abundant natural biopolymer after cellulose. In the United States, chitosan has been approved as a GRAS substance by the FDA (Anonymous 2001). It is reported to exert antimicrobial effects against different groups of microorganisms, including bacteria, molds and yeasts. Gram positive bacteria appear to be more sensitive to chitosan than are Gram negative bacteria (Rhoades *et al.* 2000; Roller *et al.* 2000; No *et al.* 2002; Xie *et al.* 2002). Chitosan derivatives that have antimicrobial properties include quaternary chitosan (N,N,N-trimethyl chiosan, N-propyl-N,N-dimethyl chitosan and N-furfuryl-N,N-dimethyl chitosan), carbohydrate-branched chitosan derivatives (1-deoxyglucit-1-yl chitosan and 1-deoxylactat-1-yl, and water-soluble chitosan (chitosan lactate and chitosan hydroglutamate) (Rabea *et al.* 2003). According to Kim *et al.* (2003), compared with lipase-degraded chitosan, O-carboxymethylated (O-CM) chitosan had a higher antibacterial effect in that low concentrations (0.01 and 0.02%) of the compound were very effective at inhibiting *S. aureus*, *B. subtilis* and *S.* Typhimurium, and 0.1 and 0.5% was effective at inhibiting *E. coli*.

Mechanisms of antimicrobial effects

The antimicrobial efficacy of chitosan has been proposed to be affected by the pH of the medium (**Fig. 1**). The main mechanism could be attributed to the soluble status of chitosan and its protonated form when pH is low. Electron microscopy studies by Helander *et al.* (2001) have shown that chitosan acts on Gram negative bacteria by disrupting the barrier properties of the outer cell membrane. Rabea *et al.* (2003) have also demonstrated that when pH of the medium is less than 6, the positively charged chitosan molecule could alter cell permeability of the bacteria by binding its positively charged amino ($-NH_3^+$) groups to the negatively charged carboxylate ($-COO^-$) group located on the outer surface of bacterial cell membranes. Further, atomic force microscopy studies of the antibacterial activity of chitosan nanoparticles (Qi *et al.* 2004) indicated that chitosan nanoparticles could induce disruption of cell membranes and leakage of the cytoplasm of *Salmonella* Cholerasuis. Also, the fact that chitosan films with cross-linking structure and low solubility in water has negligible antimicrobial effect, may support the idea that the molecule has to be soluble in order to act as an antimicrobial agent (Zivanovic *et al.* 2005; Pranoto *et al.* 2005).

The MW of chitosan has been demonstrated to be critical for its inhibitory effects (Hirano *et al.* 1989). The lower MW of chitosan leads to its greater capability of dissolving in water. Rabea *et al.* (2003) suggested that chitosan with a

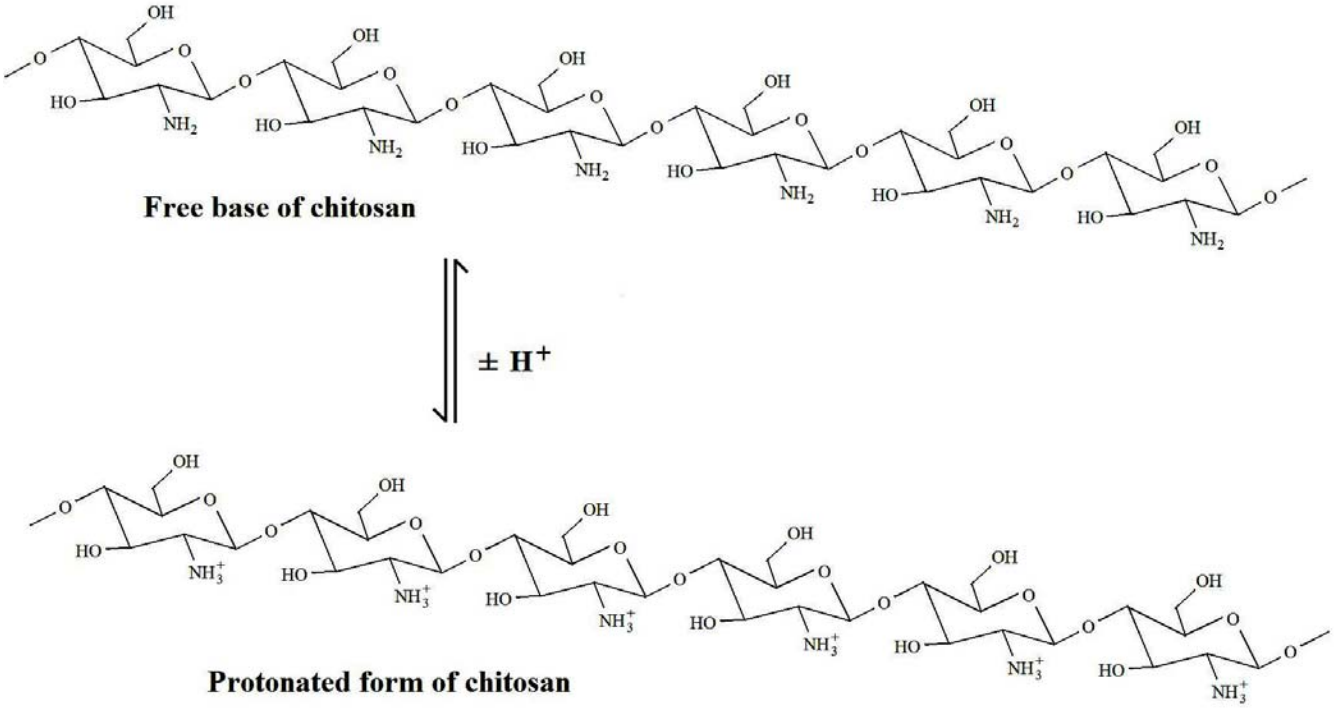

Fig. 1 Different antimicrobial mechanisms of chitosan based upon pH. The free base is postulated to involve chelation to trace elements and metalloenzymes, while the protonated form acts to disrupt cell membranes, thus inducing leakage of cell components (adapted from Juneja *et al.* 2006).

range of MWs from 10,000 to 100,000 would be desirable to restrain bacterial growth. No *et al.* (2002) concluded that chitosan with a low degree of acetylation (<15%) and a MW of 28 kDa to 1671 kDa showed the strongest antibacterial effects in aqueous solutions. According to Liu *et al.* (2001), the antimicrobial activity of chitosan increases with MWs of 5,000 to 91,600 and decreases with MWs in the range of 91,600 to 1,080,000. This has been proven by Kim *et al.* (2003), who found that degraded O-CM chitosan with a MW of 78,600 had higher antimicrobial effects than degraded chitosan of MW 30,000, while O-CM chitosan of MW 435,500 showed a lower antimicrobial effect than chitosan of MW 120,000. In such a case, a more insightful explanation would be that an increase in positive charges on the chitosan molecule leads to a higher inhibitory efficacy. In other words, the addition of O-CM to lower MW degraded chitosan leads to interaction with NH_2 group in an intra- or intermolecular way to impart a charge, while the addition of O-CM to the higher MW chitosan leads to cross-linking through strong intramolecular hydrogen bonding, thus reducing the effective number of NH_2 groups that are available to attach to bacterial surfaces (Kim *et al.* 2003). In addition, the degree of deacetylation and the concentration of chitosan in solution also play important roles in its antimicrobial effects. Furthermore, chitosan may also inhibit the production of toxins and bacterial growth by chelating trace metal ions and essential enzymes (Cuero *et al.* 1991).

Application in Meat Products

Chitosan alone

A critical review and discussion of the antimicrobial properties of chitosan has been published by Roller *et al.* (2003). This includes its inhibitory effects against a variety of pathogenic and spoilage organisms in raw minced meat products like beef patties (Darmadji *et al.* 1994), raw minced pork and skinless and standard raw pork sausages (Sagoo *et al.* 2002). Juneja *et al.* (2006) evaluated the ability of chitosan to protect cooked ground beef and turkey against *C. perfringens* spores during chilling. It was reported that 3% chitosan in ground beef or turkey may reduce the potential risk of germination and growth of *C. perfringens* spores that may occur by abusive chilling for 12, 15, or 18 h. However, the authors had "no apparent explanation" for the fact that some of the mean log CFU/g from cooked or chilled meat samples pretreated with chitosan had higher numbers than that of the control that contained no chitosan. A review by No *et al.* (2007) has briefly introduced aspects of chitosan applied in food products, including meat and sausage.

Chitosan combined with other hurdles

Low MW chitosan - chitosan solution and mixture

A combination of chitosan with reduced concentrations of traditional preservatives has shown great potential for meat preservation. A combination of chitosan and nitrite has been investigated in cured Korean sausage (Youn *et al.* 1999) where 50% nitrite (75 ppm) was substituted with 0.2% chitosan. Contrary to results from the use of nitrite alone (150 ppm) and chitosan alone (0.2, 0.35 or 0.5%), the variable total bacterial numbers in sausages containing the combination were below the detection limit of the plate counting method from day 0 to day 4, and increased to 3 log CFU/g by day 7 at 30°C.

A combination of chitosan and lactic acid also showed well-developed antimicrobial activity. According to Youn *et al.* (2004), 1.0% chitosan (MW 120 kDa, degree of deacetylation 85%) dissolved in 0.3% lactic acid, significantly reduced the total bacterial cell counts, thus, remarkably lengthening the shelf life of spicy beef.

Darmadji *et al.* (1994) reported that in meat, 0.5-1.0% chitosan inhibited the growth of meat spoilage bacteria, including *Pseudomonas*, *Staphylococcus*, coliforms, and some Gram negative bacteria at 30°C for 48 h or storage at 4°C for 10 days. Also, in liquid medium, a 0.01% concentration of chitosan inhibited meat spoilage bacteria while a higher concentration of 0.1 and 1.0% could inhibit the meat starter cultures, *L. plantarum* IAM 1216, *P. pentosaceus* IAM 12296 and *Micrococcus varians* IFO 3765. This indicated that a combination of chitosan and meat starter cultures may enhance meat preservative quality, as well as prevent over-fermentation by controlling the growth of lactic acid bacterial starter cultures.

Chitosan coating has also been shown to enhance the antimicrobial effect of vacuum packaging with respect to its inhibitory effect of anaerobic meat spoilage bacteria, specifically, lactic acid bacteria. According to Yingyuad *et al.* (2006), by dipping grilled pork samples into chitosan solutions (2.0% and 2.5% w/v commercial grade chitosan in acetic acid) before vacuum-packaging, total viable counts were reduced by 2 to 3 log CFU/g after 14 to 28 days under storage at 2°C.

The combination of chitosan, carnocin and sulfite has been studied for preservation of chilled pork sausages (Roller *et al.* 2002). This study showed that chitosan (0.6%) combined with a low concentration of sulfite (170 ppm) retarded the growth of spoilage microorganisms more effectively (3-4 log CFU/g reduction) at 4°C for up to 24 days, compared with a high concentration of sulfite alone (340 ppm). Further, sulfite degraded slower in chilled fresh pork sausages in the presence of chitosan. The mechanism of preservation by the combination of chitosan and sulfite was postulated to be two-fold: chitosan may act as a "slow-release" agent for sulfite and unbound chitosan may have selectively inhibited yeast growth, thus preventing acetaldehyde production by spoilage yeasts which can inactivate sulfite by binding it.

According to Rao *et al.* (2005), a 4 kGy irradiation treatment followed by chitosan coating (dipping into 1% chitosan solution) of shelf-stable intermediate-moisture meat products, including fresh mutton kebabs and streaky bacon, resulted in no viable bacteria or fungi detection.

A greater antimicrobial effect was demonstrated when chitosan was combined with certain plant extracts. The simultaneous addition of both 260 mg/kg rosemary extract and 10 g/kg chitosan (MW 490,000, degree of deacetylation: 88.2%, viscosity: 75 cps) to "traditional Greek sausages" (fresh pork sausages) resulted in significant and the highest inhibitory effects against meat spoilage organisms, including the Enterobacteriaceae, *Pseudomonas* spp., total viable bacteria, yeasts and molds and lactic acid bacteria (Georgantelis *et al.* 2007). A chitosan (degree of deacetylation 78~82%) and mint mixture (CM) had comparable antimicrobial effects to chitosan alone against common food spoilage and pathogenic bacteria, including *E. coli*, *P. fluorescens*, *S.* Typhimurium, *S. aureus* and *B. cereus* in minced lamb meat and pork cocktail salami, with the minimum inhibitory concentration of 0.05%. It was also shown that CM was more effective against gram-positive than Gram negative bacteria (Kanatt *et al.* 2008a).

In addition, a chitosan–glucose complex (CGC), a partial Maillard reaction product achieved by heating chitosan with glucose, was found to be identical to chitosan in inhibition against the common food spoilers and pathogens, including *E. coli*, *Pseudomonas* spp., *S. aureus* and *B. cereus* (Kanatt *et al.* 2008b).

High MW chitosan - chitosan films

The antimicrobial properties of chitosan may become negligible when chitosan is in the form of insoluble films (Ouattara *et al.* 2000). However, by adding other antimicrobial components, the chitosan films strongly inhibit pathogenic and spoilage bacteria in meat products. The use of packaging films containing antimicrobial agents also proves to be quite efficient by slowing the migration rate of the agents away from the surface, thus assisting to maintain high concentrations of the agents where they are needed (Ouattara *et al.* 2000).

Li *et al.* (2006) studied the antimicrobial effect of konjac glucomannan (KGM) edible film incorporated with chitosan and nisin against *E. coli*, *S. aureus*, *L. monocytogenes* and *B. cereus*. Their study showed that the blend film KC2-

nisin (mixing ratio of KGM 80/chitosan 20 incorporated with 463 IU nisin) and chitosan-nisin (chitosan with 463 IU nisin) exhibited significantly higher antimicrobial activity ($P > 0.05$) than KGM-nisin (KGM alone with 463 IU nisin), demonstrating that incorporating chitosan into KGM film improved its antimicrobial activity.

Pranoto *et al.* (2005) studied chitosan films (chitosan MW of 900,000 to 1000,000, degree of deacetylation, approximately 95%) incorporated with garlic oil up to levels of at least 100 µl/g, potassium sorbate at 100 mg/g or nisin at 51,000 IU/g against common meat contaminants. Agar diffusion tests showed that this film had inhibitory effects against *S. aureus*, *L. monocytogenes* and *B. cereus*, while no inhibition resulted from the use of chitosin film alone. This also supports the action model of chitosan that when its MW is between 91,600 to 1,080,000, the cross-linking of chitosan exerts no antimicrobial effects. For *E. coli* and *S.* Typhimurium, only chitosin films incorporated with garlic oil or nisin showed any contact inhibition.

By evaluating the antimicrobial effects in bologna, Zivanovic *et al.* (2005) reported that chitosan films enriched with 1% and 2% oregano essential oil decreased the numbers of *L. monocytogenes* by 4 log CFU and *E. coli* O157:H7 by 3 log CFU. On the other hand, pure chitosan film reduced *L. monocytogenes* by 1-3 logs and had no effect on *E. coli* O157:H7 after storage of the bologna slices at 10°C for 5 days.

Ouattara *et al.* (2000) studied the antimicrobial effects of chitosan-based films applied in processed meat products, specifically bologna, pastrami, and cooked ham. After 21 days under refrigerated storage, chitosan-based antimicrobial films types CA (chitosan/acetic acid), CAC (chitosan/ acetic acid/cinnamaldehyde) and CAL (chitosan/acetic acid/ lauric acid) which were applied to the meat surfaces significantly reduced ($P \leq 0.5 - 0.05$) the growth of indigenous Enterobacteriaceae in pastrami, and completely inhibited them in bologna. As for cooked ham, chitosan-based antimicrobial films CA, CAC, CAL, NCA (neutralized CA film), CP (chitosan/ propionic acid) and CPC (chitosan/ propionic acid/cinnamaldehyde) resulted in a significant reduction of *Serratia liquefaciens* during an entire 21-day refrigerated storage period.

LACTOFERRIN

Lactoferrin is a pink, globular glycoprotein (MW ca. 80 kDa, pI = 8-9) that has been found to be the main iron-binding protein present predominately in mammalian milk. Lactoferrin has great potential to be used as a natural antimicrobial in foods. This is because it possesses antimicrobial effects against a wide range of Gram positive and Gram negative bacteria, fungi, viruses and parasites (Farnaud *et al.* 2003), including the foodborne pathogens, *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* spp. and *S. aureus*, and food spoilage bacteria, such as *Pseudomonas* spp. and *Bacillus* spp. Lactoferrin is also able to detach multi-drug-resistant bacteria from biosurfaces (Naidu 2002). Moreover, it can promote the growth of probiotics, including *Bifidobacterium* spp. and *L. acidophilus* (Naidu 2000; Aguila *et al.* 2001; Kim *et al.* 2004). Lactoferrin can be produced by both recombinant DNA technology and by purification from large volumes of cow's milk or whey (Vorland 1999).

Mechanisms of Antimicrobial Effects

The main structure-functional property of lactoferrin lies in its ability to bind two Fe^{3+} ions with high affinity (Sallmann *et al.* 1999), in cooperation with two HCO_3^- ions. The ability to sequester free iron ions required for bacterial growth may contribute to its bacteriostatic effect (Oram *et al.* 1968; Weinber 1975). According to more current studies, the high-affinity interaction of lactoferrin with pore-forming outer-membrane proteins (OMPs) of Gram negative enterics, including *E. coli*, has been demonstrated as critical for its antimicrobial activity (Gado *et al.* 1991; Naidu *et al.* 1997). This has also been reflected by lactoferrin-mediated outer-membrane damage in Gram negative bacteria (Ellison *et al.* 1988) and lactoferrin-induced antibiotic potentiation by altered permeation (Naidu *et al.* 1994). Accordingly, lactoferrin could inhibit microbial attachment to subepithelial matrix proteins and detach bacteria from mucosal surfaces (Naidu 2002). Furthermore, lactoferrin may work intracellularly to block microbial attachment factors of bacteria, such as fimbriae and other adhesins (Naidu *et al.* 1997). In addition, promoted growth of any existing probiotics in the medium may also assist the inhibition of pathogenic bacteria by lactoferrin (Griffiths *et al.* 2003).

The antimicrobial activity of lactoferrin is dependent on its protein confirmation and milieu conditions (Naidu *et al.* 1997). Denaturation or structural alteration of lactoferrin has been noticed from the process of its isolation from milk (Naidu 2002). Bicarbonate (HCO_3^-) can prevent the formation of inactive lactoferrin tetramers by divalent cations. Because of this, it has been described as a "companion" anion to lactoferrin (Ellison *et al.* 1988). Also, isolated lactoferrin is highly susceptible to conformational changes, thermal uncoiling and proteolysis, as well as pH, ionic environment and improper citrate/bicarbonate ratios (Naidu 2002). It is reported that the antimicrobial effect of lactoferrin was impaired by the presence of cations, including Na^+ (Al-Nabulsi *et al.* 2005), Ca^{2+} and Mg^{2+} (Al-Nabulsi *et al.* 2006a), due to the cation stabilization of the outer bacterial membrane (Coughlin *et al.* 1983).

Application in Meat Products

Milk-derived lactoferrin is considered GRAS by the U.S. FDA (21 CFR 170.36(f)). It is permitted at levels of 65.2 mg/kg of beef, according to the FDA's Directive of October 23, 2001. The U.S. Department of Agriculture (USDA) approved the use of lactoferrin on fresh beef in December, 2001 (Naidu 2002).

The antimicrobial property of lactoferrin is not evident if the dissolved lactoferrin is simply mixed into meat during processing. Chiu *et al.* (2007) mixed 40 or 80 mg/kg lactoferrin into hot-boned (4–5 h after death) ground pork and stored them at 4°C. Although the ground pork with lactoferrin had lower ($P \leq 0.05$) total plate counts than the controls at 3, 6 and 9 days of storage, the differences in total plate counts between the two lactoferrin treatments were only significant ($P \leq 0.05$) at days 3 and 6. Further, only the addition of 80 mg/kg lactoferrin decreased lactic acid bacterial counts at days 0, 3 and 9, albeit not significantly ($P > 0.05$).

Immobilized activated lactoferrin (ALf)

To stabilize the lactoferrin structure, thus enhancing its antimicrobial property, immobilization of the molecule has been proven to be a feasible solution. Activated lactoferrin (ALf) could largely enhance the antimicrobial spectrum of lactoferrin. A patent has been developed to produce ALf wherein milk lactoferrin is immobilized on a food-grade glycosaminoglycan, such as galactose-rich polysaccharide or carrageenan solubilized in a pre-calibrated citrate/bicarbonate buffer system containing sodium chloride and an excess of unbound lactoferrin. By such processing, the immobilized lactoferrin showed an enhancement in antimicrobial effects, including microbial adhesion blocking, bacterial detachment, microbial growth inhibition, and antiviral activity (Naidu 2002). This has been well reflected by tests of ALf compared with lactoferrin against collagen-bound and beef steak-inoculated *E. coli* O157:H7 (Naidu 2002). Also, an additional 10 s-spray/rinse with 1% ALf resulted in effective decontamination of contaminated beef surfaces, not only by inhibiting bacterial growth, but also by removing debris and residual bacteria (Naidu 2002), thus removing existing toxins.

Microencapsulated lactoferrin

Research on the antimicrobial effects of microencapsulated lactoferrin in packaging films indicate its potential use for packaging cured meat. According to Al-Nabulsi (2006b), two types of microcapsules containing lactoferrin have been

studied against the meat spoilage organism, *Carnobacterium viridans*, on bologna. By applying the encapsulated molecule on the surface of bologna under vacuum at 4 or 10°C for 28 days, these authors found that the release of lactoferrin from the microcapsules were more temperature-stable for water/oil/water (W/O/W) emulsion than W/O emulsion. Further, freeze-dried W/O/W lactoferrin emulsion incorporated in edible whey protein isolate (WPI) packaging films showed greater antimicrobial activity than unencapsulated lactoferrin, along with the addition of metal chelating agents. However, when applied as an ingredient in dry cured sausage, the microencapsulated and unencapsulated lactoferrin had limited antimicrobial effects. According to Al-Nabulsi *et al.* (2007), the antimicrobial activity of encapsulated and unencapsulated lactoferrin was tested after incorporating the compounds into sausage batters during processing, and the results showed that certain strains of *E. coli* O157:H7 were only injured but not lethally inactivated (Al-Nabulsi *et al.* 2007).

LACTOPEROXIDASE SYSTEM

The lactoperoxidase system (LPS) consists of three primary components: the lactoperoxidase enzyme (LPX), thiocyanate (SCN^-) and hydrogen peroxide (H_2O_2). It occurs naturally in bovine milk (Reiter 1985) and saliva of humans and animals (Tenovuo 1985). The LPS is a GRAS substance, which allows its use as a natural food preservative.

Mechanisms of Antimicrobial Effects

The primary target of the LPS is the cell membrane (Klaenhammer 1993). It is believed that the main effect of the LPS is the oxidation of sulfhydryl groups of enzymes and other proteins by $OSCN^-$ or $(SCN)_2$, which is the intermediary oxidation products of thiocyanate (SCN^-), leading to altered cellular function, such as membrane damage, leakage of essential cell constituents, failure of transport systems, and inhibition of metabolic enzymes (Reiter *et al.* 1984; Thomas 1985; Perraudin 1991; Wolfson *et al.* 1993).

The LPS is highly temperature dependent, and shows the best antimicrobial effect at storage temperatures that are non-permissive for rapid growth of bacteria (Kennedy *et al.* 2000; Elliot *et al.* 2004). This may be further attributed to cell density and the level of cellular metabolic activity, since cellular repair mechanisms may be inefficient at a low temperature, and cells risk metabolic exhaustion if they attempt to repair damage induced by the LPS at low temperatures. The pH of meat product surfaces and incubation times may affect the antimicrobial activity of the LPS. In addition, water content has been proven to have little or no effect on the antimicrobial activity of the LPS (Kennedy *et al.* 2000).

Application in Meat Products

Although much research has been done on the antimicrobial activity of the LPS on pure culture in broth and food matrices, only a small portion of studies concentrated on meat products. It is reported that the LPS is capable of significantly inhibiting pathogenic bacteria, both Gram positive and Gram negative, in meat products but it is not as effective against native or spoilage lactic acid bacteria.

Wolfson *et al.* (1994) studied LPS on poultry and found that in conjunction with a thermal heat treatment, this antimicrobial showed inhibition not only against *Salmonella*, but also against spoilage psychrotrophic bacteria. In addition, Tan *et al.* (2006) demonstrated that adding the LPS at a level of 1 unit (1 µg/ml LP, 5.9 mM KSCN and 2.5 mM H_2O_2) significantly ($P \leq 0.05$) decreased the total microflora and psychrotrophic counts of marinated broiler drumsticks, especially for samples that received a prior thermal treatment at 58°C for 2 min.

Kennedy *et al.* (2000) conducted the earliest research to evaluate the antimicrobial effects of the LPS against pathogens on red and ground meats. According to their study, high concentrations of the LPS (200 mg/kg meat) were necessary to inhibit *E. coli* O157:H7 by 6 log CFU at 12°C after five days, *L. monocytogenes* by 6 log CFU at 6°C after 15 days, and *S. aureus* by 5 log CFU at 12°C after eight days in ground beef. Also, with the exception of one fecal coliform, growth of the native microbial populations in ground beef was strongly inhibited by the LPS at room temperature for 4 h, which represents a situation of severe temperature abuse.

In addition, by studying microbial inhibition in beef cubes under 12°C, 37°C and a typical commercial meat operation chilled temperature regime, Elliot *et al.* (2004) concluded that low concentrations of the LPS (up to 10 mg LPS/kg beef) with temperatures non-permissive for rapid bacterial growth was effective at inhibiting pathogenic bacteria, including *S. aureus*, *S.* Typhimurium, *L. monocytogenes*, *E. coli* O157:H7, *Yersinia enterocolitica*, and *Pseudomonas aeruginosa*. However, it failed to inhibit spoilage lactic acid bacteria at chilling temperatures.

Also, different combinations of the LPS with lipids, including palmitoleic acid, monopalmitolein, lauric acid, caprylic acid, and sodium lauryl sulphate (McLay *et al.* 2002), and bacteriocins have been studied. Although these combinations showed an enhancement in the antimicrobial activity of the LPS on meat-related pathogens and spoilage bacteria pure cultures, they were less effective when applied in meat products.

McLay *et al.* (2002) found that in Todd Hewitt Broth (THB), the combinations of 5-200 mg/kg LPS with 50-1000 ppm monolaurin were best at inhibiting the growth of *E. coli* O157:H7 and *S. aureus*. However, in ground beef, these combinations failed to enhance the inhibition of *E. coli* O157:H7 over the use of the LPS alone. In contrast, the combination did reduce more of the growth of *S. aureus* in ground beef at 6 h, compared with the LPS alone. The mechanism of bacterial inhibition by the LPS-monolaurin combination is still not known. A synergistic effect of the inhibition of *L. monocytogenes* has also been reported for LPS used in combination with nisin (Zapico *et al.* 1998).

MONOLAURIN

Monolaurin (glycerol monolaurate), a glycerol monoester of lauric acid (medium-chain fatty acids), has profound antimicrobial activity. It has also gained approval for use as a food emulsifier by the U.S. FDA (Oh and Marshall 1992).

Mechanism of Antimicrobial Effects

It is believed that the cell membrane is the primary target of monolaurin. Because of its lipophilic property, monolaurin can disrupt membrane integrity which, in turn, interferes with membrane activities, such as transport of amino acids that then leads to cell starvation (Kabara 1993), as well as inactivation of enzymes and/or genetic functions (Branen *et al.* 1980). Monolaurin has a more significant antimicrobial activity against Gram positive than Gram negative bacteria, including the meat-related pathogens, *L. monocytogenes* (Wang *et al.* 1992; McLay *et al.* 2002) and *S. aureus* (Kabara *et al.* 1972), and meat-related spoilage bacteria, such as *B. thermosphacta* and *L. mesenteroides*, but not other lactic acid bacteria (Blaszyk *et al.* 1998). The antimicrobial activity of monolaurin can be enhanced by low pH (Oh *et al.* 1992, 1993) and temperatures (Wang *et al.* 1992; Oh *et al.* 1993), and changes in the redox potential of the medium (Kabara 1984). Also, synergistic interactions occur when this antimicrobial is combined with other natural agents. It is reported that the antimicrobial spectrum and activity of monolaurin increased in the presence of organic acids (Kato *et al.* 1976), such as sorbic acid (Kabara 1984), and sodium citrate (Blaszyk *et al.* 1998). In addition, Blaszyk *et al.* (1998) demonstrated that monolaurin could positively interact with eugenol and other essential oils (pimento oil, thyme, horseradish oil and allylisothiocyanate), as well as sodium citrate, to inhibit meat spoilage and pathogenic bacteria, including *L. sakei*, *L. curvatus*, *L. mesenteroides*, *B. thermosphacta* and *E. coli* O157:H7, although the lactic acid bacteria and *E. coli* O157:H7 were more resistant than the

others (Blaszyk *et al.* 1998).

Application in Meat Products

Monolaurin (500 µg/g) caused a delayed growth of *L. monocytogenes* at 5°C, although its efficacy was quite limited in that approximately 5-$\log_{10}$ increase in the number of the pathogen was observed in refrigerated cooked meat products during 45 days of storage (Mbandi *et al.* 2004). When applied in meat products, the fat may absorb monolaurin, thus lowering its effective biocidal concentration (Robach *et al.* 1981). This may explain why studies on the antimicrobial activity of monolaurin in meat products have utilized monolaurin concentrations of as high as 3000 to 5000 ppm (Kabara 1984). The combination of monolaurin with other antimicrobial agents, such as the LPS (McLay *et al.* 2002), could enhance its antimicrobial effect, as well as lower its effective concentration.

BACTERIOPHAGES

The use of foodborne bacteriophages is a promising new way to more specifically target certain pathogenic bacteria. Such bacteriophages are more specific to pathogenic bacterial targets, yet harmless to mammals. In August, 2006, the U.S. FDA announced the approval of a bacteriophage preparation, LMP-102^{TM}, which was submitted by Intralytix Inc. (Baltimore, MD), as a food additive to be used on RTE meat and poultry products to inhibit *L. monocytogenes*. This preparation is composed of six naturally-occurring bacteriophages isolated from the environment (FDA CFSAN, 2006). More recently, in October, 2006, FDA and USDA extended the GRAS approval status for LISTEX^{TM} P100 bacteriophage produced by EBI Food Safety (Wageningen, the Netherlands) for use on all food products susceptible to *Listeria*, which is composed of one specific bacteriophage against *L. monocytogenes* strains (http://www.ebifoodsafety.com/en/news.aspx, 2006).

Intralytix, Inc. has also manufactured another bacteriophage-based antimicrobial product that is effective against *E. coli* O157:H7, designated ECP-100^{TM}. The evaluation of this product by the U.S. FDA started in April, 2007, and is still ongoing (http://www.intralytix.com/ Intral_Food.htm, 2007). Further, EBI Food Safety is also working on bacteriophages against *Salmonella* and *Campylobacter jejuni* with the intent that these products can be applied in chicken meat in the future.

CONCLUSIONS AND FUTURE PERSPECTIVES

The microbial safety of meat and diversely processed meat products has always been a key concern in the meat industry. Traditional meat preservatives, such as salt and nitrites, although effective against foodborne pathogens and spoilage bacteria, have been shown to lead to long-term ill effects in health, such as cardiovascular diseases and cancer. Further, consumers are looking for more natural alternatives to foods containing chemical preservatives. As a result, novel natural antimicrobial agents, which are inhibitory towards undesirable foodborne microorganisms, yet safe to be ingested by humans without residues or health concerns, have been increasingly studied for their application to food. In this review paper, we have mainly concentrated on promising natural antimicrobial agents studied in meat and meat products, including bacteriocins, chitosan, lactoferrin, monolaurin, lactoperoxidase system, and more recently, bacteriophages. Among these antimicrobials, most of them are still under study and development and not all are approved to be used in foods. In order to be applied in the meat industry, more research is needed on these and other potential antimicrobial natural compounds with respect to their effectiveness at promoting the shelf life of meat products, their performance in conjunction with other antimicrobials that are inherent in meats or added to meats, and their tolerance to various meat processing steps and procedures.

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