Natural Antimicrobials, Parasitic Bacteria and Bacteriophages

INTERVENTION SUMMARY	
Status	Currently available
Location	Packaging/retail
Intervention type	Surface treatment or mixed into product, or impregnated into product packaging
Treatment time	Part of product, or product packaging
Regulations	Some oil extracts approved in the EU, US and Australia Bacteriocins (e.g., nisin) approved in the US and Australia (under consideration in the EU)
Effectiveness	Has Potential, if used as part of whole of chain microbial reduction approach
Likely cost	Variable
Value for money	Cost of extraction can be expensive
Plant or process changes	Not likely to be a huge impact as can be incorporated into processing e.g., impregnated into packaging, included in product mixing etc
Environmental impact	Minimal
OH&S	None identified
Advantages	Some oil extracts may add a flavour benefit to the meat product, e.g., rosemary, garlic, cloves
Disadvantages or limitations	Cost of extraction of some antimicrobials may be too expensive for some applications Some food components may inhibit the bacteriocins May limit markets due to changing product characteristics, e.g., flavour

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There has been an increasing recognition of the potential application of natural antimicrobial agents in foods. Numerous studies have reported antimicrobial properties of extracts and essential oils of certain plants. A group of bacteria has been found to produce peptides or proteins that are inhibitory to other bacteria. There are also bacteria that prey on others, and bacteriophages (bacterial viruses). All of these have shown some promise as potential interventions to extend storage life and reduce the risk of food poisoning.

Plant Extracts

Plant extracts and essential oils have received a lot of attention for use in meat products due to their antioxidant and antimicrobial activities as well as flavour properties. They could be found in various parts of plants, including leaves (e.g., rosemary and oregano), flowers or buds (clove), bulbs (garlic and onion), seeds (fennel and parsley), and fruits (pepper) (Zhu *et al*., 2005; Ahn *et al*., 2004). It has been reported that plant extracts and essential oils are more effective against Gram-positive (e.g., *Staphylococcus*, *Listeria* spp. and lactic acid bacteria) than Gram-negative bacteria (e.g., *E. coli*, *Pseudomonas* and *Salmonella*) (Chen *et al.,* 2012).

Extensive research has been carried out to evaluate the potential use of essential oils as antimicrobial agents in packaging. In a recent study, application of 0.1% of thyme essential oils was found to reduce microbial population by up to 2.8 log cfu/g in modified atmosphere packaged lamb meat after 9 days of storage at 4°C (Karabagias *et al.,* 2011). Another investigation has reported that a 1.12 log reduction of *E. coli* O157:H7 level was observed on whole beef muscles that were coated with bioactive films containing 1% oregano essential oils after 7 days of storage at 4°C (Oussalah *et al.,* 2004).

Microbial Products

Bacteriocins are natural antimicrobial agents produced by bacteria. They can be applied during the processing of raw meat, or cooked meat products before packaging, to prevent growth of spoilage microorganisms or food-borne pathogens (Chen *et al.,* 2012).

Nisin is one of the most commonly used and investigated bacteriocins for food preservation. Its use is approved in the US and Australia in processed meat products. In the US, a blend of encapsulated nisin preparation (90.9%), rosemary extract (8.2%) and salt (0.9%) is also approved for use in frankfurters and other similar cooked meat and poultry sausages.

The antimicrobial activity of nisin has been evaluated, and nisin generally appears to be more effective against Gram-positive than Gram-negative bacteria. Cutter and Siragusa (1995) have demonstrated that spraying inoculated beef carcass surfaces with nisin reduced *Brochothrix thermosphacta*, *Carnobacterium divergens*, and *L. innocua* ranging from 1.8 to 3.5 log units. However, application of nisin under commercial conditions only produced little effects (<0.2 log) on uninoculated beef carcass surfaces (De Martinez *et al.*, 2002).

The cost of extraction of natural antimicrobials can make them expensive particularly when used in complex food systems, and the bactericidal activity can be inhibited by binding of the bacteriocins to food components and inactivation by enzymes such as proteases (Ganzle *et al*., 1999).

A number of lactic acid bacteria (LAB) have been shown to inhibit pathogen growth in ground beef. Specifically, *Lactobacillus reuteri* is a highly effective competitive inhibitor to *E. coli* O157:H7 in ground beef stored under modified atmosphere packaging, and has been responsible for reductions of up to 6 log units during 20 days storage (Muthukumarasamy *et al.*, 2003). *Lactobacillus plantarum* can also reduce the population of *E. coli* O157:H7 by 1.5 log units and *Salmonella* by 3 log units when added to ground beef before vacuum packaging. A cocktail of four strains of LAB added to ground beef was found to be effective in reducing *E. coli* O157:H7 and *Salmonella* by 3 log cycles in overwrap and MAP packs (Hoyle *et al*., 2009). Taste panels indicated that there were no detrimental effects on the ground beef after 5 days storage with the lactic acid bacteria, while significant reductions in the numbers of *E. coli* O157:H7 and *Salmonella* were also observed in the product (Smith *et al.,* 2005).

Bacteria that naturally produce bacteriocins can be added to cooked meat products as starter cultures, before packaging, to inhibit growth of spoilage organisms.

Parasitic Bacteria

Parasitic bacteria, especially *Bdellovibrio bacteriovorus* prey on a range of Gram-negative pathogens and spoilage organisms (Hanlin and Evancho, 1992). These organisms are present in soil and faecal contents of many species, and can be isolated and purified. Little work has been done on their applications to foods, but *Bdellovibrio* isolates have achieved 2.5-7.9 log reductions in *E. coli* and *Salmonella* populations during 7 hours in culture, and 3.0-3.6 log reductions on stainless steel (Fratamico and Cooke, 1996), over a period of 24 hours. The organism is most effective at 30-37°C, but between 12 and 19°C, parasitism still occurs, but more slowly (Fratamico and Whiting, 1995).

Bacteriophages

Bacteriophages or phages are the viruses of the microbial world. They only attack and destroy their host microorganisms. Bacteriphages are a natural antimicrobial and are considered as safe for use in food. Several studies have evaluated the potentials for using bacteriophages to prevent the growth of spoilage and pathogenic organisms in a wide range of foods (Greer, 2005). However, because of their host-specificity, their application is limited in which a single bacteriophage against one bacterial strain might not be effective against another. The effectiveness of bacteriophages as antimicrobial agents is also limited by factors such as potential resistance development by host bacteria (Greer 2005).

The use of bacteriophages has been approved by the USDA as cattle hide wash. A scientific opinion by the European Food Safety Authority (EFSA, 2009) reviewed the use and mode of action of bacteriophages, and concluded that there was insufficient evidence that they protect against recontamination with bacterial pathogens.

Bacteriophages could be used to treat farm animals or animal products, such as carcasses (Sillankorva *et al*, 2012). In a recent trial, Hudson *et al.* (2012) have demonstrated that bacterialphage 'FAHEc1' at >10^7 PFU/ml caused a 4-log reduction of *E. coli* O157:H7 at 5 °C in broth. However, when the same bacteriophage (at 3.2×10^7 PFU/4 cm^2) was applied on beef pieces under conditions simulating hot boning and conventional carcass cooling, inactivation of *E. coli* O157:H7 of approximately 2 log was observed.

Proponent/Supplier Information

Further information on natural antimicrobials including nisin and protective bacterial cultures can be obtained from:

Danisco Australia Pty Ltd

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Botany, NSW 2019

Australia

Phone: 02 9384 5000

Website: http://www.danisco.com.au/

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