

Meat Safety News Digests

A collection of recent news relevant to the safety of red meat prepared by the Food Safety Program of Meat & Livestock Australia, for SAFEMEAT Stakeholders

STEC DETECTION

Use of the *ecf1* gene to detect the presence of STEC in beef samples

Standard testing methods using *stx*, *eae*, and O serogroup-specific gene sequences for detecting the top six non-O157 STEC (i.e., O26, O103, O121, O111, O145, and O45) typically possess the disadvantage in which these genes may reside, independently, in different non-pathogenic organisms, leading to false-positive results. To this end, researchers from the U.S. explored the utility of the *ecf* operon as a single marker to detect *eae*-positive STEC from pure broth and primary meat enrichments. During the initial study, analysis of 501 *E. coli* isolates demonstrated a strong correlation (99.6%) between the presence of the *ecf1* gene and the combined presence of *stx* and *eae* genes. Two subsequent studies were carried out to evaluate the potential use of an *ecf1* detection assay to detect non-O157 STEC strains in enriched meat samples in comparison to the results using the U.S. Department of Agriculture Food Safety and Inspection Service (FSIS) method that detects *stx* and *eae* genes. It was evident in ground beef samples ($n = 1,065$) that the top six non-O157 STEC were detected in 4.0% of samples by an *ecf1* detection assay and in 5.0% of samples by the *stx*- and *eae*-based method. In contrast, in beef

samples composed largely of trim ($n = 1,097$), the top six non-O157 STEC were detected at 1.1% by both methods. Estimation of false-positive rates among the top six non-O157 STEC revealed a lower rate using the *ecf1* detection method (0.5%) than using the *eae* and *stx* screening method (1.1%). These results highlight that *ecf1* detection assay has the potentials to be used as an alternative method for detection of non-O157 STEC to the standard methods.

<http://www.ncbi.nlm.nih.gov/pubmed/25836391>

INTERVENTION

Mechanisms of inactivation of *E. coli* O157:H7 after exposure to hot water treatment

Several studies have demonstrated that antimicrobial treatments consisting of hot water sprays alone or paired with lactic acid rinses are effective for reducing *E. coli* O157:H7 loads on beef carcass surfaces. However, the mechanisms by which these interventions inactivate bacterial pathogens are still poorly understood. The objective of this U.S. study was to test the hypothesis in which *E. coli* exposed to hot water become more susceptible to lactic acid due to an increase in deterioration of bacterial outer membrane lipids. Cocktails of *E.*

coli O157:H7 strains were subjected to hot water at 25 (control) 65, 75, or 85°C incrementally up to 60 s. Surviving cells were then enumerated by plating, and formation of lipid-based compound from bacterial membranes was quantified using a spectrophotometer. Inactivation of *E. coli* after a hot water exposure was found to be duration- and temperature-dependent manner, with populations being reduced to non-detectable numbers following heating of cells in 85°C water for 30 s. Formation of lipid-based compound was, however, not dependent upon increasing water temperature or exposure period. These results suggest that hot water application causes degradation of membrane lipids, which may increase the sensitivity of *E. coli* to subsequent lactic acid application.

<http://www.ncbi.nlm.nih.gov/pubmed/26038913>

Effects of high-pressure treatment on cold-shocked *E. coli* O157:H7 in beef-based broths

Researchers from Canada examined the pressure resistance of the stationary phase and the exponential phase *E. coli* O157:H7 in 0.1% peptone water, beef gravy and ground beef, upon exposure to low temperatures. The sub-lethal injury of this organism and the baro-protective role of those three food systems were also assessed. Stationary phase cells and exponentially-growing cells were cold-shocked (10°C). Cold-shocked and non-cold-shocked stationary phase *E. coli* were subjected to pressure treatment at 400 MPa for 20 min at 30°C, whereas exponential phase cultures were treated at 200 MPa for 8 min at 20°C. Quantitative estimates of sub-lethal injury to *E. coli* cells were then

performed using the differential plating method. The results revealed no significant differences ($P > 0.05$) in the reduction of cell numbers between cold-shocked or non-cold-shocked cultures. This indicates that cold shock did not confer cross protection for both stationary phase and exponential phase cells to high-pressure treatment. However, cold-shock treatment appeared to increase the sub-lethal injury to cells cultured in peptone water (stationary and exponential phases) and ground beef (exponential phase), but decreased the sub-lethal injury to cells in beef gravy (stationary phase). Cells grown in ground beef (stationary and exponential phases) appeared to experience lowest death compared with other types of broth. This suggests that baro-protective effects are greater in real food systems than model food systems, indicating the need for using real food systems in establishing food safety parameters for high-pressure treatments.

<http://onlinelibrary.wiley.com/doi/10.1111/jam.12794/abstract>

MEAT SPOILAGE

Identification of meat spoilage gene biomarkers in *Pseudomonas*

Pseudomonas plays an important role in the spoilage of meat products under refrigeration and aerobic conditions. Identifying genes regulated by *Pseudomonas* under different environmental conditions would provide a better understanding of how this organism adapts to current packaging and storage conditions. To this end, European researchers examined the gene expression profile of *Pseudomonas putida* grown at different temperatures

(30°C and 10°C) and decreasing glucose concentrations (from 0.04 to 0.00 mg/ml), to identify key genes actively involved with the spoilage process. The data revealed differences in the level of expression of genes between the two temperatures. These differences were, however, higher at initial glucose concentrations and considerably lower when glucose was close to depletion, suggesting that glucose concentration plays a more important role than temperature in determining bacterial gene expression. Furthermore, some of the genes with changes in their expression (between 10°C and 30°C) were found to contribute to meat spoilage. These included those genes that are involved in metabolic pathways and the production of specific metabolites (e.g., malodourous compounds).

<http://www.sciencedirect.com/science/article/pii/S0956713515002042>

Use of neem cake extract to control meat spoilage organisms

The focus of this Italian study was to evaluate the antibacterial activity of an ethylacetate neem cake extract (NCE) against bacteria that affect meat quality, namely *Campylobacter jejuni*, *Carnobacterium* spp, *Lactobacillus curvatus*, *Lactobacillus sakei* and *Leuconostoc* spp. The antibacterial activity of NCE at 1 mg/ml was determined using standardised disc diffusion and macro-dilution methods. The results revealed a significant difference ($P > 0.05$) between the inhibition zone of NCE and control. The percentage of bacterial growth reduction ranged from 79% to 91% when compared with control. These indicate

that NCE could be used to suppress the growth of meat spoilage organisms.

<http://www.tandfonline.com/doi/abs/10.1080/14786419.2014.964708#.Vcl6OvmqpBc>

SPECTROSCOPY

Predicting meat quality traits of lamb *m. semimembranosus* using hand held Raman spectroscopic device

This Australian study determined the potential of a hand held Raman spectrometer to measure lamb quality traits other than tenderness for the first time. Complementary trials were conducted to predict meat quality traits of fresh lamb *m. semimembranosus* (topside) after ageing and freezing/thawing. Raman spectroscopic measurements were conducted on 160 randomly selected lamb carcasses (80 fresh muscles at 24 h and 5 d post-mortem, another 80 at 24 h, 5 d and following freezing/thawing). Shear force, cooking loss, sarcomere length, colour, particle size, collagen content, pH₂₄, pH_u, purge and thaw loss were also measured. Results showed the potential to predict pH_u, pH₂₄ and L^* measured at 1 and 5 d post-mortem on fresh intact lamb from Raman spectra observed at 1 d post-mortem. Further, spectra collected at 5 d post-mortem could predict purge as well. However, no ability to predict shear force even after freezing and thawing was observed.

<http://www.sciencedirect.com/science/article/pii/S0309174015300371>

Prediction of total viable counts in beef by hyperspectral imaging

The aim of this study was to investigate the practicality of hyperspectral scattering imaging to predict the low levels of bacterial contamination in beef stored at 4°C for 12 days. The visible/near-infrared hyperspectral images were acquired from 3-5 beef samples on each day of the experiment, in parallel with enumeration of TVC population. Parameters representing different hyperspectral image characteristics were extracted and used to establish the statistical models for predicting beef TVC using different modelling methods. Results showed that the models developed using individual parameters did not perform well in predicting low levels of TVC contamination in beef. However, better modelling results were achieved when parameter combination approach was adopted. This study demonstrated for the first time that hyperspectral imaging supported with appropriate data analysis tools could be used to detect low levels of bacterial contamination in beef non-destructively.

<http://www.sciencedirect.com/science/article/pii/S0260877415001594>

LAB

Lactic acid bacteria and their controversial role in fresh meat spoilage

In this review the authors' appraised meat associated lactic acid bacteria (LAB) and their role in meat preservation and spoilage. Adaptation of LAB to storage conditions such as vacuum and modified atmosphere packaging and thereby oxidative stress

and ability to proliferate competitively under a large range of gas combinations have been demonstrated. Cold-chain maintenance and avoidance of temperature abuse during handling, transport and storage exert a selection pressure that proved favourable for LAB growth and proliferation. Possible contamination sources such as processing environment and diversity of LAB (cold acclimatized mesophiles and strictly psychrotrophic species) are discussed. Adaptation of *Leuconostoc gelidum* as a spoilage organism in packaged refrigerated meat and meat products has been reported. Different spoilage-associated molecules such as volatile organic compounds produced by LAB are identified.

Authors emphasised the controversy surrounding the role of LAB in fresh meat spoilage. The spoilage characters of some LAB taxa are ambiguous and probably correlated with specific spoilage-associated capacities of individual bacterial strains of a species that cannot be ascribed communally to the whole species. Some LAB strains have bio-protective function in meat as they can provide favourable antagonistic activity against other undesired microorganisms. Further, authors acknowledge that the study and interpretation of LAB spoilage in meat is a complicated and challenging phenomenon and in order to be defined, in depth understanding of its complexity beyond species level is demanded, as well as combination of different disciplines and tools.

<http://www.sciencedirect.com/science/article/pii/S0309174015001114>

LISTERIA

Effect of acidified sorbate on *Listeria monocytogenes* on meat surfaces

Researchers in this study examined and quantified the lag-phase durations (LPD) and growth rates (GR) of *L. monocytogenes* on the surfaces of cooked sliced ham as affected by different sorbate concentrations (0 – 4% wt/v) and pH (4.0 – 6.5) at refrigerated and abuse temperatures (4 – 12°C). Slices of cooked ham inoculated with a four-strain mixture of *L. monocytogenes* (ca. 10^3 CFU/g) were surface treated with sorbate solutions of different pH, vacuum-packaged and stored for 45 days. Response models were developed using LPD and GR. Results showed that the growth of *L. monocytogenes* on ham was inhibited for >45 days with treatments of 4% sorbate solutions at pH 4.2 and 4.5 stored at 8 and 6°C, respectively. The models estimated the LPDs of *L. monocytogenes* were 0 to 11 days and the GRs were 0.25 to 0.36 log CFU/day, respectively at pH 4.0 to 5.5 (no sorbate) when stored at 4°C. The LPD prolonged to 2 to 26 days and 34 to >45 days, and the GRs were reduced to 0.15 to 0.30 and 0 to 0.19 log CFU/day, respectively with the treatments of 2 and 4% sorbate solutions. Further, increasing sorbate concentrations by 1% to 2, 3, and 4% at pH 5.5 to 4.0 led to an extension of LPD by 2 to 11, 10 to 19, and 18 to 27 days, whereas the GRs were reduced by 0.04 to 0.06, 0.05 to 0.07, and 0.06 to 0.08 log CFU/day, respectively at 4°C. The study showed that sorbate concentration and pH level were significant factors affecting the LPD and GR of *L. monocytogenes* and that the combination of sorbate and low pH has potential for use as a surface

post-processing antimicrobial treatment to control *Listeria* on meat surfaces.

<http://www.ingentaconnect.com/content/iafp/jfp/2015/00000078/00000006/art00013>

NITRATE/NITRITE

Implications of reducing nitrate and nitrite levels in dry-fermented sausages

This Spanish study evaluated the effect of reducing the concentration of nitrate and nitrite on the quality of *chorizo*, traditional high-acid (pH < 5.5) dry-fermented sausages. The sausages were prepared using different concentrations of nitrate/nitrite; 150 mg/kg KNO₃ and 150 mg/kg NaNO₂ (high nitrate/nitrite batch), 112.5 mg/kg KNO₃ and 112.5 mg/kg NaNO₂ (25% reduction), 75 mg/kg KNO₃ and 75 mg/kg NaNO₂ (50% reduction) and a control batch with no nitrate/nitrite. The typical microbiota (lactic acid bacteria, Gram-positive catalase-positive cocci and *Enterobacteriaceae*) and the residual levels of nitrate/ nitrite in sausages were analysed at day 0, 3, 14 and 27 of ripening. Volatile analysis was performed at the end of ripening.

The results showed that the concentration of nitrate/nitrite significantly affected Gram-positive catalase-positive cocci, where numbers were 1 and 2 log CFU/g higher in the 50% reduction and control batches, respectively. *Enterobacteriaceae* increased during fermentation at lower nitrate/nitrite concentrations. Whereas, the amount of volatiles derived from amino acid degradation and carbohydrate fermentation increased at lower nitrate/nitrite levels. A relationship

was found between ingoing and residual nitrite, which was 3.5 fold higher when the maximum amount was used in comparison to the 50% reduction. However, future reduction of the maximum ingoing levels of nitrate/nitrite in dry fermented sausages should be studied in the context of a risk benefit balance between the chemical and microbiological hazards.

<http://www.sciencedirect.com/science/article/pii/S0956713515002418>

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FOR FURTHER INFORMATION PLEASE CONTACT:

Manager, Market Access Science and Technology

Ian Jenson

PH: 02 9463 9264

ijenson@mla.com.au



MEAT AND LIVESTOCK AUSTRALIA, PO Box 1961 NORTH SYDNEY NSW 2059