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Comparison of Selective Media Overlays for the Enumeration of Heat-Injured *Enterococcus Faecium* from Nuts

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Abstract

Food processing steps generally involve exposing bacterial contaminants to treatments such as heating, freezing, acid stress, ultraviolet irradiation, or osmotic shock. It is recommended that the food industry validate these processes to ensure the destruction of pathogenic microorganisms such as *Salmonella* spp., *Listeria monocytogenes* and *Bacillus cereus*. Process validation procedures which include a microbiological challenge test involve direct inoculation of food samples with a non-pathogenic bacterial surrogate, in-plant processing of the food sample and subsequent microbiological enumeration to determine the effectiveness of the 'kill-step' in reducing the pathogen of concern. The repair and recovery of the injured bacterial cells after sample treatment is extremely important to avoid an overestimation of the inactivation effects of the 'kill step'. Injured bacteria may not readily grow on selective media, and non-selective enrichment steps preclude the enumeration of the original density in the food sample. Recovery of the injured cells after sample treatment can be enhanced by using non-selective media (for samples with very low background microorganisms) or plate count media overlaid with selective media. This study compares two selective media overlays, Bile Aesculine Azide agar and Kenner Faecal Streptococcus agar, for the enumeration of *Enterococcus faecium* (a surrogate for *Salmonella Enteritidis* PT30) from roasted nuts. Inoculum preparation and sample inoculation was conducted following the method developed for inoculation of almonds for process validation (Almond Board of California, 2014). The selective media overlay method was used for microbiological enumeration of roasted nuts. Both media overlays recovered densities significantly higher than selective media. This study may allow for more accurate laboratory plating and the interpretation of thermal process validation microbiological data.