TOOLS FOR MICROBIAL DETECTION

DETECTION OF *SALMONELLA* SPP. BY PCR IN POULTRY LITTER

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Salmonella spp. are important foodborne pathogens in the United States and can be disseminated through the food chain via poultry products, by-products and poultry litter. Poultry litter can be used as a soil amendment. Prompt identification of this microorganism in poultry litter by a reliable and rapid method is important to establish an effective program for controlling *Salmonella*. This study determined the optimal pre-enrichment and enrichment incubation conditions and detection limits for PCR to detect *Salmonella* in poultry litter. The detection capability of PCR was compared to culture methods using 15 *Salmonella enterica* serotypes. As PCR targets, *sdi*A and *hil*A genes were used. The results were compared by calculating the kappa indexes at 95% CI. The shortest combination times for the pre-enrichment and enrichment steps which led to positive results by PCR were 6 h in 0.1% peptone water and 18 h in Rappaport-Vassiliadis broth, respectively. The calculated detection limit for the PCR tests was 10 *Salmonella* CFU/100 g of litter. The aforementioned time schedule and calculated detection limit were selected for further analysis with the 15 *Salmonella* strains. There was no significant difference in the PCR identification of *Salmonella* when either *sdi*A or *hil*A were used as targets ($p < 0.0001$). When PCR and the conventional methods were compared, they disagreed and this disagreement was statistically significant ($p < 0.05$). PCR was not able to detect *S.* Agona while *S.* Montevideo and *S.* Typhimurium were not re-isolated by the conventional microbiological method. The results of these experiments suggest that shorter incubation times might lead to false negative results in PCR tests. The analysis of multiple samples at longer incubation times for the enrichment step might increase PCR sensitivity and reduce the disagreement between tests.

INSTRUMENTAL HEADSPACE ANALYSIS FOR ASSESSMENT OF SPOILAGE IN PACKAGED AND STORED RAW SALMON

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Over the years, a great number of methods have been proposed for assessing fish spoilage. Despite the intensive effort to find a rapid and reliable microbiological, chemical, or instrumental method, assessment of freshness or spoilage in the seafood industry has traditionally relied on trained panels or trained inspectors to judge the quality of fish during refrigerated storage. While trained panels have previously been employed to measure spoilage of inoculated *Clostridium botulinum* fish samples, these panels were limited in the number of panelists (2-5 members) due to the constraints that individuals be immunized. More recently, the FDA Institutional Review Board, who reviews studies involving human participants as subjects, has ruled that even immunized trained panelists should not be permitted to smell botulinum inoculated fish samples due to the potential risks of inhaling aerosolized spores or toxin. These imposed constraints require that spoilage studies of *C. botulinum* inoculated fish samples be monitored by chemical or instrumental analyses. Currently within our lab, studies are addressing the potential for toxin development to precede spoilage in a high-fat fish product, salmon, when packaged using films of varying permeabilities (oxygen transmission rates (OTR) of 3, 3,000, 10,000, and 15,000 cc/m^2/24 h at 24°C), different atmospheres (air, vacuum, and modified atmosphere (MA)), and different storage temperatures (4, 8, 12, and 16°C). Consequently, a critical component to these *C. botulinum*-inoculated studies has been to use an ultra-fast gas chromatography instrument called the Znose®, to collect, separate, and quantify individual headspace volatiles, in an effort to identify one or more chemical markers that could be associated with spoilage. In a follow-up study, uninoculated raw salmon was stored for varying periods and presented to a consumer panel to correlate quantities of each volatile peak with their acceptance or rejection of the product.

Several volatiles were generated during storage of *C. botulinum*-inoculated and uninoculated raw salmon, but the dominant volatiles present varied with the package atmosphere, storage temperature, storage time, and presence of *C. botulinum*. The dominant volatile produced under aerobic conditions for uninoculated salmon samples stored at 4, 8, and 12°C had a Kovats Index (KI) of 753 and was tentatively identified as 2- or 3-methyl 1-butanol. No difference in quantity of this volatile occurred during 4 or 16°C aerobic storage of salmon packaged with the 4 different OTR films. Film permeability did significantly affect the size of the KI-753 volatile

peak during vacuum and MA storage at 4, 8, and 16°C with the smallest quantities in each atmosphere being produced in salmon packaged with the non-permeable film (OTR of 3). Interestingly, *C. botulinum*-inoculated vacuum samples at both 4 and 8°C had reduced generation of the KI-753 volatile.

Two other volatile peaks were also generated under aerobic conditions and had KIs of 640 and 720. The latter peak was tentatively identified as 3-hydroxy-2-butanone and appeared to become more dominant when samples were stored at higher temperatures. Several peaks (KIs of 806, 975, 1200 and 1465) were evident only when salmon was stored at 12 or 16°C with greater quantities of KI-975, KI-1200, & KI-1465 volatiles being generated during storage for samples packaged in lower OTR films. For inoculated samples stored at 12 and 16°C, a peak with a KI of 688 dominated for most film types under either vacuum or MA.

In the follow-up storage study, 53 elderly consumers (60-85 years of age) participated in a consumer panel by evaluating 20 uninoculated raw salmon samples stored for varying periods of time. Using a hedonic scale, overall, appearance, and aroma acceptance of each product was judged by the panelists. In addition, panelists were asked whether they would prepare the product in their kitchen given they had already purchased the product. Following evaluation by the panelists, the headspace of these samples was analyzed using the zNose and areas of 12 peaks were compared to acceptance ratings. A regression model incorporating both the KI-753 and KI-640 volatiles was significant and accounted for 56% of the variability in the aroma scores. Aroma and overall scores of 2-3 (dislike very much – dislike moderately) did not translate into unwillingness to prepare the sample for up to 7 panelists. Based on their household practices, however, only one of those panelists would ever store the fish for more than one day and hence could likely encounter extremely spoiled fish. In summary, two key volatiles were related to consumer perception. Very high levels of those volatiles were necessary before all panelists would reject the sample.

RAPID DETECTION OF ORGANOPHOSPHATE OF PESTICIDE RESIDUES FROM TEA USING SERS WITH A SILVER NANOROD ARRAY SUBSTRATE

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Tea is a popular beverage in the United States, however, recently food safety issues have arisen regarding pesticide residues on tea. To address this concern, a simple, rapid, and sensitive method to detect the pesticide is necessary. To achieve rapid determination of pesticide, the use of surface-enhanced Raman spectroscopy (SERS) with Ag nanorod array substrate produced by oblique angle deposition (OAD) method at vapor incident angle of 86° was investigated. Conventional gas chromatography method was used for confirmation. Chlorpyrifos, an organophosphate pesticide used for many crops and plants including tea, was studied at a laser excitation wavelength of 785 nm. The feasibility of SERS detection technique with the specific Ag nanorod array substrate to rapidly examine pesticide residues in tea was demonstrated. We showed that there was a quantitative relationship between the concentration of chlorpyrifos and the SERS peak intensities. Calibration curves based on Raman areas at bands 419, 691, 1343, and 1575 cm^{-1} had better correlation coefficients (0.9609-0.9901) than that based on Raman intensity at 691, 1343, and 1575 cm^{-1} (0.9285-0.9731) in a chlorpyrifos concentration range of 0.2-100 ppm. The use of SERS spectra for detection of chlorpyrifos left on off-shelf tea samples was successfully demonstrated. Principal component analysis (PCA) was objectively used to confirm false or positive pesticide residues. Our results suggest that the SERS combined with PCA can be used to identify pesticide residues in food systems via identifying the minute different fingerprints.

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