

## FISH/SHELLSFISH

### FATE OF *SALMONELLA* IN NUTRIENT BROTH AND ON THE SURFACE OF RAW OYSTERS AS AFFECTED BY CHITOSAN

(W. Klypradit, P. Chhabra, and Y. W. Huang)

This study was initiated to determine the effect of chitosan, a modified carbohydrate polymer derived from chitin, on growth inhibition of *Salmonella* spp. as tested in nutrient broth and in shelled oysters. Three levels of chitosan, 1, 2, and 3%, were prepared in 0.5% acetic acid and the pH adjusted to 6.5 by adding NaOH solution. Pure cultures of *Salmonella* were inoculated into nutrient broth containing the chitosan in acetic acid or applied to oysters coated with the chitosan/acetic acid material. All nutrient broth samples were kept at 7°C for a period of 48 h while oysters were kept at 4°C for 15 d. Stored inoculated broths and oysters were sampled at 12-h and 5-d intervals, respectively. Initial counts of all broth samples averaged  $6.0 \times 10^8$  cfu/ml. A 7- $\log_{10}$  reduction occurred in the presence of chitosan/acetic acid after 48 h incubation at 7°C while samples containing 0.5% acetic acid alone exhibited a 2-3  $\log_{10}$  reduction. The initial counts in oysters averaged  $1.8 \times 10^5$  cfu/g. After a 5-d storage period, *Salmonella* populations started to decline. After a 15-d storage period, *Salmonella* populations declined 1, 3, and 2- $\log_{10}$  in oysters exposed to 1, 2, and 3% chitosan, respectively. No reduction in *Salmonella* population was observed in oysters coated with an acetic acid solution. Overall, 2% chitosan was considered the best treatment level for inactivation of *Salmonella* on raw oysters.

### SURVIVAL OF SELECTED LACTIC ACID BACTERIA IN THE BUFFER OF PACKAGED MUSSELS (C. M. Lin, Z. Yan, J. L. Kornacki, and M. P. Doyle)

Modified atmosphere packaging (MAP) is a commonly used practice in the food industry to increase shelf life. However, naturally occurring *Clostridium botulinum* in MAP foods may produce a life-threatening toxin(s) before the products are spoiled and rejected by consumers. This project was undertaken to determine the suitability of selected strains of lactic acid bacteria (LAB) for future competitive exclusion experiments to prevent *C. botulinum* in live mussels. LAB strains were screened for their ability to survive wide variations in pH, oxygen content and storage temperature in a commercial buffer solution, with and without added mussels, used to package the mussels.

Six LAB cultures were tested for growth at aerobic and anaerobic conditions on TSAYE and acidified MRS media and were also evaluated on McLung's media (a medium commonly used to recover *C. botulinum*) using an ecometric technique. All six LAB strains grew well under aerobic and anaerobic conditions.

Changes in mussel buffer pH in the presence of mussels were monitored at 3°C and 12°C, i.e., temperatures at which mussels may be stored subsequent to packaging. Mussels were shipped overnight from the manufacturer and 25 mussels (1.25 lb) were added to 500 ml of mussel buffer. The pH increased from 2.5 to 4.0 at both temperatures 1 h after combining live mussels with buffer. The pH was 4.5 at 3 h and nearly neutral at 24 h. There were no differences in pH values of mussel preparations at both temperatures at equivalent sampling times.

Changes in pH were monitored in buffer after the addition of LAB and live mussels with and without 1% glucose. Three LAB were selected and grown in MRS broth for 48 h, then 3 ml was added to 300 ml of buffer with 10 mussels. Addition of glucose did not affect the pH and the presence or absence of glucose did not influence the ability of LAB to change the pH of the buffer.

Survival of the three LAB strains in buffer with added mussels was determined. The initial cell numbers evaluated were:  $1 \times 10^9$  cfu/ml for strain 43201;  $1.6 \times 10^8$  cfu/ml for strain 11454; and  $4.8 \times 10^6$  cfu/ml for strain 43200. LAB were enumerated on acidified MRS agar (pH 5.4). Populations of all LAB strains declined to the lowest level 2 h after they were mixed with the buffer and remained unchanged thereafter. Results revealed that populations of LAB strains were reduced in the buffer, but there was substantial survival of LAB in buffer with living mussels for 24 h. LAB strains 43201, 11454, and 43200 were reduced at 24 h by approximately 2.5, 3, and 1  $\log_{10}$  cfu/ml, respectively.

To determine survival of a selected LAB strain in the mussel package under actual processing conditions, LAB strain 43201 was shipped to the manufacturer, and added to mussel buffer used for packaging. Strain 43201 was grown in MRS broth at 37°C for 24 h and added into the mussel buffer at ca.  $1 \times 10^6$  cfu/ml. The packages were

shipped to the Center for Food Safety immediately and stored at 3°C and 12°C upon receipt. The bacterial populations were monitored at 4, 7, and 11 days after packaging. Total aerobic plate counts, LAB counts, and pH values were monitored. Results revealed that LAB strain 43201 survived at ca.  $1 \times 10^5$  cfu/ml throughout 11 days at both temperatures. No LAB cells were detected on MRS agar in control samples of uninoculated mussels. Total aerobic plate counts were significantly higher than controls at 7 and 11 days after packaging. There were no significant pH changes in buffer with added LAB and uninoculated control at 4 and 7 days after packaging, but at day 11 a lower pH was observed in the LAB treated packages. Temperatures did not substantially affect survival of LAB; however, total aerobic plate counts were higher at 12°C than at 3°C. Mussels in packages held at 12°C had an undesirable odor after 7 days but not at 3°C.

In summary, selected LAB strains grew well under both aerobic and anaerobic conditions. The pH of buffer with/without LAB strains increased to near neutrality within 24 h after addition of live mussels. The addition of glucose did not have a significant effect within 24 h on pH or survival of LAB strains in buffer. The maximum population decline of selected LAB strains occurred within 2 h after their addition to buffer with mussels. LAB strain 43201 survived at ca.  $10^5$  cfu/ml for up to 11 days after packaging in buffer with mussels when initially inoculated at  $10^6$  cfu/ml under actual processing conditions. Storage of mussels in buffer with LAB strains did not change the odor of mussels held at 3°C. Studies are planned to determine the ability of selected LAB strains to inhibit the growth of *C. botulinum* in mussels packaged in buffer.

This research demonstrated potential survival of selected lactic acid bacteria in a proprietary mussel packaging system. The inhibition effect of LAB strains on *C. botulinum* in mussel packages will be tested in the next phase of this work.

