Quality Characteristics of Fresh Blue Crab Meat Held at 0 and 4°C in Tamper-Evident Containers

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ABSTRACT

There has been a regulatory movement toward the required use of tamper-evident containers for fresh blue crab meat. North Carolina passed tamper-evident regulations in 1993. Blue crab processors had little information on possible changes in head-space gases, microbial growth, chemical decomposition, sensory quality, or shelf life caused by the new containers. Chemical, microbiological, physical, and sensory changes in fresh crab meat were monitored during 18 days of storage in ice and 13 days of storage refrigerated at 4°C. “Special” blue crab meat, chosen for the study, is the least expensive commercial form of white crab meat. The crab meat was packaged in four retail containers: copolymer polyethylene cups with polyethylene snap-on lids, copolymer polyethylene cups with snap-on polyethylene lids fastened to the cup with heat-shrink low-density polypropylene seals, copolymer polyethylene cans with aluminum easy-open ends, and copolymer polypropylene cups with a tamper-evident pull-tab on the lid. Control samples packaged in industry standard copolymer polyethylene cups maintained higher oxygen levels than meat stored in tamper-evident containers. No consistent differences in quality or shelf life were detected among the containers. Market shelf life was limited to 6 days for meat held at 4°C and 15 days for meat held at 0°C. Sensory quality deteriorated 6 days earlier for crab meat held at 4°C than meat held at 0°C. Collateral work showed that toxin production by Clostridium botulinum neither occurred following 18 days of storage at 4°C nor after 15 days of storage at 10°C. Definite spoilage occurred before any toxin production. The study suggests that blue crab processors can safely use the new tamper-evident packaging, which has little or no effect on product quality or shelf life. Processors may choose appropriate packaging options using price, packaging quality, market appearance, and ease of production as the deciding criteria.

Key words: Bacteria, blue crab meat, headspace gases, iced, refrigerated, seafood, sensory analyses, storage, tamper-evident package

On 1 April 1993, North Carolina regulators at the Department of Environment, Health, and Natural Resources announced that all blue crab meat sold or processed within the state must be packaged in tamper-evident containers. The regulators were addressing health concerns about consumers opening crab meat containers to examine the contents before purchasing the product. Industry concerns were related to economic fraud in the marketplace. Tamper-evident containers were recommended to prevent the switching of container lids identifying a higher-priced product form with lids from a lower-priced product form. State authorities approved several general types of tamper-evident packaging. Similar requirements have been adopted, are pending, or are being considered for action in other states.

This investigation was initiated to learn the consequences of new tamper-evident packaging on the quality and safety of fresh blue crab meat held either iced or refrigerated. Little or no information was available to the blue crab industry, regulatory officials, or packaging manufacturers detailing the effects of the tamper-evident containers on head-space gases, microbial growth, chemical decomposition, sensory quality, or shelf life of fresh blue crab meat. The probability of toxin production by Clostridium botulinum at refrigeration temperatures also needed to be evaluated. Crab processors and regulators needed this basic information to assess the safety and effectiveness of the new containers.

METHODS

Harvesting, cooking, and picking of blue crabs

Blue crabs, Calinectes sapidus, were harvested commercially for the study. Live crabs were collected in crab pots from Georgia waters, transported by truck to the cooperating blue crab processing plant, and cooked under commercial conditions in a pressurized steam retort. The crabs were cooked on the afternoon of the same day that they were harvested to an internal temperature ≥112.8°C. The retorted crabs were placed in a walk-in refrigerator at ≤7.2°C and held until the next morning. Crab meat was removed from the body cavity by hand using commercial processing methods. The meat was picked into standard plastic cups, which were weighed, closed, and then iced. Total picking time was approximately 4 h. Chilled crab meat was then transferred to the experimental and
control containers used for both the refrigeration and in-ice conditions of the storage study.

Packaging and storage conditions

"Fresh" crab meat is defined as cooked meat removed from the crab's body cavity or claws that is then held iced or refrigerated without further processing. Further processing could include pasteurization to produce pasteurized crab meat held in ice or refrigerated at less than 1.7°C. Fresh "special" blue crab meat was chosen for the study. "Special" meat is the least expensive commercial form of white crab meat removed from the body cavity of cooked crabs. This product form includes smaller white pieces or chunks of body meat and generally excludes more expensive backfin meat. Backfin meat refers to large chunks or pieces of white meat taken from areas adjacent to the crab's backfin (16). Chemical, microbiological, physical, and sensory changes in fresh special blue crab meat were monitored during 18 days of storage in ice at 0°C and for 13 days of storage in refrigeration at 4°C. Both iced and refrigerated meat were held in a walk-in refrigerator. The iced sample containers were placed in small portable coolers and covered completely with ice. Melt-water was allowed to drain continuously from the coolers. Ice was added as needed to maintain complete coverage of the control and experimental retail containers.

The meat was packaged in four types of retail containers: (i) industry standard 12-oz copolymer polyethylene cups with polyethylene snap-on lids that do not show evidence of tampering (control container) (Virginia Design Packaging Corporation, Suffolk, VA); (ii) 12-oz copolymer polyethylene cups with heat-shrink tamper-evident low-density polypropylene seals; a hand-held heat gun was used to shrink the seals to form a tight fit (Virginia Design Packaging); (iii) 10-oz copolymer polyethylene cans with aluminum easy-open ends (King Plastic Corporation, Orange, CA); lids were sealed to the cans with a Dixie Canner Can Sealer (Dixie Canner Equipment Company, Athens, GA); and (iv) 12-oz copolymer polypropylene cups with an integral tamper-evident pull-tab top (Tri-Plas Incorporated, Monroe, NC).

Fresh special blue crab meat was packed into 30 containers of each experimental and control package type. Three packages in ice of each container type were randomly selected for sampling on storage days 3, 6, 9, 12, 15, and 18. Refrigerated (4°C) packages were sampled at 3, 6, 9, and 13 days of storage. An initial composite of fresh meat used to pack the four cup styles for both 0°C and 4°C storage served as the zero-day sample for all container types.

Chemical, microbiological, physical, and sensory analyses

Oxygen and CO$_2$ levels were measured from the headspace of each intact container using an Illinois Instruments 3600 headspace analyzer (Ingleside, IL). Crab meat quality was estimated through chemical, microbiological, sensory, and physical analyses of a three-container composite sample for each of the package types on every sample day. Ammonia levels (19) and pH (10) were measured with specific ion electrodes. A meat/deionized water slurry that was diluted 1:10 was used for both pH and ammonia determinations. Total volatile base nitrogen (TVB-N) levels were 26.2 mg of NH$_3$ per 100 g (control) to 91 mg of NH$_3$ per 100 g (aluminum-end cans). The Tri-Plas pull-tab containers had significantly greater mean ammonia levels (43 mg of NH$_3$ per 100 g) than the cups with the heat-shrink seals (37 mg of NH$_3$ per 100 g) on day 12. The pull-tab cups had significantly higher ammonia levels (97 mg of NH$_3$ per 100 g) than the control (63 mg of NH$_3$ per 100 g) and the heat-shrink-sealed cups (61 mg of NH$_3$ per 100 g) samples on day 15. Mean ammonia levels increased rapidly for all container types between 12 and 18 days of storage.

There were no significant differences in pH levels among the four sample containers. Initial meat pH was 8.1 for all containers. Final pH levels ranged from 8.06 (heat-shrink sealed cups) to 8.17 (King Plastic aluminum-end cans). Ammonia levels on day 0 were 29 mg of NH$_3$ per 100 g of meat. Ammonia levels gradually increased between 6 and 12 days of storage. Ammonia levels on day 18 ranged from 66 mg of NH$_3$ per 100 g (control) to 91 mg of NH$_3$ per 100 g (aluminum-end cans). The Tri-Plas pull-tab containers had significantly greater mean ammonia levels (43 mg of NH$_3$ per 100 g) than the cups with the heat-shrink seals (37 mg of NH$_3$ per 100 g) on day 12. The pull-tab cups had significantly higher ammonia levels (97 mg of NH$_3$ per 100 g) than the control (63 mg of NH$_3$ per 100 g) and the heat-shrink-sealed cups (61 mg of NH$_3$ per 100 g) samples on day 15. Mean ammonia levels increased rapidly for all container types between 12 and 18 days of storage.

Total volatile base nitrogen (TVB-N) levels were 26.2 mg of TVB-N per 100 g on day 0. Total volatile base nitrogen increased gradually after nine days of storage reaching 28.6, 33.4, 34.1, and 35.5 mg of TVB-N per 100 g by day 18 for the control, the pull-tab, the heat-shrink-sealed, and the aluminum-end containers, respectively. Levels in the control sample were significantly less than all other samples on day 18. Rapid increases in TVB-N levels generally coincide with bacterial spoilage in seafood. Levels above 30 mg of TVB-N per 100 g are considered an indicator of spoilage in fish (15). However, as discussed in the following sections, there were no significant differences in related sensory or microbiological evaluations of the packaged crab meat. The reduced TVB-N levels could reflect the greater gas permeability or breathability of the unsealed standard containers when compared to the sealed tamper-evident containers.

Oxygen levels decreased with time and varied consider-
ably among the four iced containers. Levels in the control samples ranged between approximately 20% and 3% O₂. Oxygen levels in the other containers dropped to less than 1% by the end of the study. On day 3 the control samples had significantly greater O₂ levels than the other cups. The pull-tab cups contained significantly less oxygen than any other containers on day 3. Oxygen levels in the pull-tab cups were lower than those found in the control samples on day 6. No significant differences were measured for days 9, 12, and 15. On day 18, the control samples contained more oxygen than the aluminum-end and Tri-Plas pull-tab containers (Figure 1). Carbon dioxide levels increased with time. Levels in the aluminum-end cans were significantly greater than those found in the control cups on days three and 18. The heat-shrink-sealed containers had greater CO₂ levels than the control and the Tri-Plas pull-tab containers on day 3. Carbon dioxide levels in the pull-tab cups were lower than those found in the control samples on day 6. No significant differences were measured for days 9, 12, and 15. On day 18, the control samples contained more oxygen than the aluminum-end and Tri-Plas pull-tab containers (Figure 1). Carbon dioxide levels increased with time. Levels in the aluminum-end cans were significantly greater than those found in the control cups on days three and 18. The heat-shrink-sealed containers had greater CO₂ levels than the control and the Tri-Plas pull-tab containers on day 9 (Figure 1). The measured O₂ and CO₂ levels support theory of the reduced gas exchange properties of the tamper-evident containers when compared to the standard industry cups.

Aerobic plate counts were not consistently different among crab meat samples taken from the four commercial containers over 18 days of iced storage. Plate counts increased rapidly after 9 days of storage. All meats exceeded 10⁶ colony-forming units (CFU)/g by day 15, reaching the end of their microbiological market shelf life (Figure 2) (8).

An aerobic plate counts increased with time, most rapidly between six and 18 days of storage. No consistent differences among the four storage containers were observed (Figure 2). Psychrotrophic plate counts showed no consistent differences among the containers during storage in ice (Figure 2). Reduced oxygen levels in the tamper-evident containers caused no apparent differences in the growth rates
and final numbers of bacteria found in the tamper-evident containers when compared to the standard industry cups. Although Hunter L or whiteness values were lower for meat collected from the aluminum-end cups through 15 days of storage, the differences were not statistically significant. The initial L value for all meat was 77.10. Mean L values were 77.22 (heat-shrink), 77.73 (tamper-evident tab), 75.85 (control), and 74.25 (aluminum-end) after 15 days of storage. Hunter a or relative redness and Hunter b or relative blueness levels showed no relevant differences. Calculated whiteness index values showed no consistent differences among meat held in the four retail containers in ice throughout the study (7, 18). However, the WI calculated for meat taken from the control cups (27.65) was significantly greater than the WI for meat in the aluminum-end cans (18.65) at 15 days of iced storage. Meat from the pull-tab cups had a greater whiteness index (23.99) than meat in the aluminum-end containers (15.99) at 18 days of storage. No other differences in the whiteness index were noted.

The sensory panel found no subjective differences in meat color or appearance among the four sample containers. Panel analyses found no consistent significant differences among the four container types for ammonia, sour, putrid, or crab odors. However, the crab meat sensory assay results showed a typical spoilage pattern. Ammonia odors increased with time, showing the most rapid increase between 12 and 15 days of storage. The most prominent sour odors developed between 12 and 18 days of storage. Putrid odors also showed the most rapid rise between 12 and 15 days of storage. Putrid odors peaked on day 15 for all samples except the heat-shrink containers, which reached their maximum putrid level on day 18. Fresh crab odor declined gradually and inconsistently over the first 12 storage days. Sensory ratings for crab odor fell rapidly between days 12 and 18. The sensory quality of iced samples began a marked deterioration at 12 days of storage.

Several monitored parameters had significant correlation coefficients with storage time. The results were statistically significant at the 0.05 level and had correlation coefficients greater than 0.7. Correlation coefficient values separated the packages into three distinct groups. The observations may be related to the change from aerobic to predominantly anaerobic conditions in the tamper-evident containers. Group 1 included psychrotrophic plate counts (0.956), aerobic plate counts (0.919), and oxygen levels (−0.886) which had the highest correlation coefficients with storage time. The second group, which encompassed combined percentage of carbon dioxide (0.738), aerobic plate counts (0.812), and ammonia concentrations (0.797), correlated well with storage day for all containers. Group 3, putrid odors recorded from control packages, correlated well with storage time (0.702). Carbon dioxide levels, all plate counts, and ammonia levels increased with time for meat from all of the containers, suggesting aerobic bacterial activity. Decreased oxygen levels support the same conclusion. The correlation values suggest by-products of bacterial reproduction and metabolism. Putrid odors are usually associated with aerobic spoilage during iced or refrigerated storage of seafood. Microflora dominated by *Pseudomonas* and *Aeromonas putrefaciens* are believed to produce many of the odorous compounds. Anaerobic storage, dominated by *Enterobacteriaceae*, *Aeromonas*, and *Lactobacillus* spp. produces fewer putrid odors (4, 13).

**Refrigerated storage**

As in the results from storage in ice, there were no consistent pH differences among the refrigerated containers. However, meat pH levels decreased between 0 and 9 days of storage for all container types. The levels began at 8.64 on day zero and reached low values of 7.54 (pull-tab), 7.55 (heat-shrink-sealed), 7.57 (aluminum-end), and 7.63 (control) containers on day 9. Final pH levels on day 13 were 8.06, 8.11, 8.12, and 8.17 for heat-shrink, pull-tab, control, and aluminum-end containers, respectively.

Initial ammonia levels of 36 mg of NH₃ per 100 g began increasing between six and 13 days of storage. Day 13 ammonia levels were 311 mg of NH₃ per 100 g (aluminum-end), 328 mg of NH₃ per 100 g (pull-tab), 362 mg of NH₃ per 100 g (heat-shrink sealed), and 529 mg of NH₃ per 100 g (control). On day 6 the aluminum-end cans (53 mg of NH₃ per 100 g) had greater ammonia levels than the other containers: pull-tab cups (37 mg of NH₃ per 100 g), control cups (35 mg of NH₃ per 100 g), and heat-shrink-sealed cups (32 mg of NH₃ per 100 g). The control cups had significantly higher ammonia concentrations (260 mg of NH₃ per 100 g) than the Tri-Plas pull-tab containers (145 mg of NH₃ per 100 g) on day 9. Control ammonia concentrations were greater than all other containers by day 13. The differences in ammonia levels may reflect the results from two separate processes, container gas permeability and microbiological action. The initial ammonia increase found on day 6 in the meat from the aluminum-end cans may have been caused by reduced container breathability. Increased aerobic microbial growth and metabolic rates are the probable sources of higher ammonia levels measured for the control cups on day 13.

Total volatile base nitrogen concentration was 26.2 mg of TVB-N per 100 g on day 0. TVB-N increased rapidly between 6 and 13 days of storage, ranging from 23.8 to 117.2 mg of TVB-N per 100 g. Final TVB-N levels on day 13 were: 117.2 (aluminum-end can), 115.9 (pull-tab), 108.1 (control), and 106.7 (heat-shrink) mg of TVB-N per 100 g of meat. The aluminum-end cans had a higher concentration of TVB-N than all other packages on day 6, at 33.2 mg of TVB-N per 100 g (23.8, heat-shrink; 25.6, pull-tab; and 26.8, control). TVB-N levels in the aluminum-end cans (58.6 mg of TVB-N per 100 g) and the control cups (60.9 mg of TVB-N per 100 g) were significantly greater than those found in the other containers on day 9 (45.1, pull-tab and 41.6, heat-shrink). TVB-N concentrations exceeded levels which suggest spoilage in fish, 30 mg of TVB-N per 100 g, on day 6 for the aluminum-end cans and for all other containers by day 9 (15).

Oxygen levels decreased with time and again varied considerably among the four containers. Levels in the control cups ranged between approximately 18% and 7.5% O₂. Oxygen levels in the other containers approached zero during storage. Both observations suggest greater breathabil-
ity of the control containers. The results support the observations made during storage in ice. Refrigerated control samples had higher oxygen levels than the other containers on all sample days and the levels were significantly greater on day 6 (Figure 3). Carbon dioxide levels increased with time. Levels in the control cups were significantly greater than those concentrations found in the other containers on days 9 and 13 (Figure 3). Greater carbon dioxide levels suggest higher aerobic metabolic and reproductive rates exhibited by bacteria growing on meat held in the control cups.

The aluminum-end plastic cans had the highest aerobic plate counts on days 3 and 6. Plate counts from the control samples were significantly greater than other samples on days 9 and 13. All samples exceeded 10^6 CFU/g by day 6, reaching the end of their refrigerated shelf life (Figure 4). Anaerobic plate counts and aerobic plate counts produced similar trends. The aluminum-end plastic cans had the highest anaerobic counts on days 3 and 6. Counts from the control cups were definitely greater than from other samples on day 13 (Figure 4). Psychrotrophic plate counts showed no consistent differences among the four package types (Figure 4). As seen in the storage in ice, differing CO_2 and O_2 concentrations did not affect the total number of bacteria recovered from meat stored in the four commercial containers.

Hunter L or whiteness levels were 74.75 on day zero for all samples and ended on day 13 with levels of 77.14 (control), 76.81 (pull-tab), 76.43 (aluminum-end), and 74.51 (heat-shrink). Aluminum-end cans had the lowest Hunter L values or whiteness levels on days 3 (73.56), 6 (74.12), and 9 (74.01). Aluminum-end L values were significantly less than control and Tri-Plas pull-tab cups on days 6 (76.63, control and 76.32, pull-tab) and 9 (78.96, control and 76.80, pull-tab). Control samples were significantly whiter than the
Tri-Plas cups on day 9. Hunter $L$ values showed the control samples to be whiter than the heat-shrink sealed products on day 13. Hunter $a$ and Hunter $b$ measurements revealed no consistent differences among the containers. Whiteness index values showed no significant differences among the four meat containers on any storage day.

As with the iced samples, the sensory panel revealed no significant differences among the refrigerated sample containers. The sensory results showed a typical refrigerated storage spoilage pattern. Ammonia odor multiplied most rapidly between day 6 and day 9. Exhibiting a similar trend, sour odor increased between 6 and 9 days of refrigerated storage. The panel's assessments of putrid sample odors were similar to the previous sensory results from the product in ice. Crab odor decreased over time, but most swiftly between day 6 and day 9. The panel's sensory evaluation of the packaged crab meat held at 4°C showed quality deterioration beginning at 6 days of storage.

Refrigerated storage produced correlation coefficients relating the measured parameters and storage days that were similar to values calculated for the iced crabmeat. However, the higher temperature (4°C) caused differences in several variables. Carbon dioxide correlation coefficients were not significant, but TVB-N levels correlated well with time. The 4°C microbiological determinations had the highest correlation values with storage time. Correlation coefficients were: 0.928, 0.913, and 0.894 for anaerobic plate counts, psychrotrophic plate counts, and aerobic plate counts, respectively. Total volatile base (0.857), ammonia (0.845), and oxygen ($-0.703$) levels also correlated significantly with storage time. Putrid odor did not correlate well with storage time for combined samples. Control (0.769), heat-shrink (0.713), and aluminum-end (0.703) containers developed relatively high individual putrid odor correlation coefficients. Meat packed in control cups also had high correlation values for ammonia (0.768) and crab odor ($-0.751$). The higher refrigeration temperatures increased the rates of bacterial metabolism and reproduction when compared to 0°C storage. Increased TVB-N concentrations and significant correlation coefficients that showed the relationship between TVB-N levels and storage time support the conclusion that bacterial growth rates were greater at 4°C than at 0°C.

Safety considerations

Harrison's collateral work (9) looked for toxin production by *Clostridium botulinum* during inoculated pack studies at 4°C and at the abusive temperature of 10°C. No toxin was detected following 18 days of storage at 4°C or following 15 days of storage at 10°C. The crab meat was obviously spoiled at 18 and 15 days, respectively. Crab meat packaged in tamper-evident containers appears to offer no greater threat for the production of toxin by *Clostridium botulinum* type E than refrigerated fresh crab meat stored in traditional retail cups.

Industry quality implications

Sensory ratings showed consistent declines in quality beginning at 12 days of iced storage. The declining sensory ratings followed previous rapid bacterial growth initiated on day 9 for aerobic plate count organisms and day 6 for anaerobic and psychrotrophic organisms. Marked oxygen depletion occurred between days 12 and 15 for the control samples and days 9 and 15 for the tamper-evident samples.

The panel's sensory evaluation of the packaged crab meat held at 4°C showed quality deterioration beginning at 6 days of storage. Microbiological analyses found 6 days to be the market shelf life of the meat. Aerobic and anaerobic plate counts began to increase between 3 and 6 days of storage. Psychrotrophic plates counts increased rapidly between day 0 and day 9. Oxygen levels decreased most rapidly following day 3. Carbon dioxide levels showed a gradual increase from day 6 for all samples but the control. The CO$_2$ headspace concentrations in the control containers quickly rose between day 6 and day 9, an indicator of increased bacterial growth. TVB-N levels began to increase rapidly between 6 and 9 days of refrigerated storage and continued to increase through the end of the study. TVB-N exceeded concentrations consistent with fish spoilage at 6 days for meat stored in aluminum cans and at 9 days of storage for all other containers. As in the iced samples, microbial growth preceded sensory quality deterioration and oxygen reduction.

Storage in ice in any of the four commercial containers investigated increased the microbiological market shelf life of the meat by 9 days compared to storage at 4°C. Storage in ice maintained good sensory quality for an additional 5 to 6 days compared to sensory quality observed during refrigerated storage.

Control samples packaged in industry standard copolymer polyethylene cups maintained higher oxygen levels than meat stored in tamper-evident containers. However, no consistent differences in quality or shelf life were detected among the four container types during iced or refrigerated storage. Market shelf life was limited to 6 days for meat held at 4°C and 15 days for meat held at 0°C. Sensory quality deteriorated 6 days earlier for crab meat held at 4°C when compared to meat held at 0°C. New tamper-evident packaging options have little or no effect on blue crab meat quality or shelf life in spite of significant differences in oxygen and carbon dioxide levels between traditional and tamper-evident containers. Price, packaging quality, market appearance, and ease of production and not quality and safety should be the major criteria used to select tamper-evident or standard packaging. It should be noted however, that the National Blue Crab Industry Association does not sanction the use of hermetically sealed containers for the commercial packaging of fresh blue crab meat.

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REFERENCES


