ARTICLES

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Role of Package Type on Shelf-life of Fresh Crab Meat

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ABSTRACT

Microbiological quality of fresh (not pasteurized) crab meat stored at 4°C in a 340 g (12 oz) food grade polyethylene traditional snap-lid container and equally fresh crab meat stored at 4°C in a 227 g (8 oz) SimpleStep® tray sealed with Cryovac™ film (oxygen transmission rate of 10,000 cc/m^2/24 h) was evaluated over a 12-day period. Aerobic plate counts were conducted on storage days 0, 4, 6, 8, 10 and 12. Anaerobic plate counts were conducted on storage days 0, 5 and 12. Aerobic plate counts of crab meat from the two containers did not differ ($P > 0.05$). Analysis of anaerobic microbial growth indicates that sampling days were significant ($P < 0.05$), but container type or style was not significant ($P > 0.05$). Oxygen and CO_2 in the package headspace was significantly different between container types ($P < 0.05$). Gas concentration between sampling days was not significant ($P > 0.05$). Results of this study demonstrate that there were no significant differences in refrigerated shelf life of crab meat packaged in SimpleStep® trays with Cryovac™ film versus the traditional polyethylene snap-lid container packaging ($P > 0.05$).

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INTRODUCTION

In the United States, competition from crab meat imports has adversely impacted the fresh crab meat industry. The Virginia Marine Resources Commission estimates that Virginia's yearly crab harvest has been decreasing since 1995 *(21)*. Several contributing factors have influenced the decline of the blue crab in the Chesapeake Bay area, including a decrease in the number of blue crabs available in the Chesapeake Bay, a decline in the ecological health of the Chesapeake Bay, and a dramatic rise in the importation of crab meat from Asia *(15)*.

Imported crab meat directly competes with domestic crab meat and is sold to local restaurants at cheaper and more predictable prices *(21)*. The combination of abundant supply, low labor cost, and growing demand for crab meat have all contributed to the popularity of imports, forcing a number of large domestic producers out of business *(11)*. New packaging could boost U.S. sales and give the domestic sellers an edge. Prior to the introduction of a new package style, research studies should be conducted to evaluate container head-space gases, microbial growth and shelf life, chemical decomposition, sensory quality and possibility of toxin production by *Clostridium botulinum (8)*.

The bacterial flora on crabs reflect the environment from which they were harvested; the flora may change from season to season depending on the water quality, water temperature and harvest location *(5)*. The flora are also influenced by environmental factors such as temperature, packaging and duration of storage *(5)*. Fresh (unpasteurized) crab meat is usually hand picked, with no further processing, which contributes to higher bacterial numbers than pasteurized crab meat *(22)*. Furthermore, fresh crab meat is a perishable product that will undergo spoilage and flavor loss within 10–14 days or less during storage *(22)*. Under refrigeration, spoilage of seafood occurs because of growth of psychrotrophic bacteria such as *Pseudomononas* spp. *(20)* and *Achromobacter (2)*. A storage temperature of 4.4°C or lower is recommended for refrigerated, microbiologically sensitive products *(6)*. The shelf life of crab meat depends on several contributing factors, including initial microbial counts and container integrity *(18)*.

New food packaging technologies can improve the quality and safety of food commodities. Packaging not only acts as a barrier for food products, but also can control the growth of microorganisms already present in the food when it is packaged. Polyethylene and polypropylene are rigid or semi rigid acrylic plastics approved for food contact. Fresh blue crab meat in the Chesapeake Bay area and the Virginia coast area is sold in traditional plastic (polyethylene) snap-lid containers of 8 oz., 12 oz. or 16 oz. Pasteurized crab meat is sold in metal cans of 8 oz., 12 oz. or 16 oz. plastic snap-lid containers sealed with metal pop top lids. Plastic and aluminum, commonly used to package crab meat, give longer shelf lives and better sensory and microbial qualities than crab meat packaged in steel cans *(9)*. It has been found that vacuum skinned packaging can improve sensory qualities of freshly cooked and picked crab meat *(9)*.

The FDA has a minimum standard of 10,000 cc/m^2/24 h oxygen transfer rate (OTR) for fresh seafood *(7)*. The transmission rate allows the transfer of gas generated from food and the outside environment, preventing the generation of potentially harmful bacteria *(4)*. Cryovac™ produces an OTR film that is an oxygen permeable film and complies with the FDA's Fish and Fisheries Products Hazard and Control Guidance (Third Edition) *(4)*. Cryovac™'s film is designed to maintain freshness and color of food products without employing CO_2 treatments *(4)*. The benefits of packaging crab meat in a polypropylene SimpleStep® tray with Cryovac™ 10,000 OTR film include innovative convenience features, such as being microwavable, easy opening, reusable and resealable *(18)*.

The new packaging also has the potential to maintain quality and safety of crab meat while providing smaller portion sizes for a broader consumer base *(9)*. Smaller, thinner packages or pouches, boil-in-bag packages and molded trays and cups can significantly increase the heating and cooling rates of their contents, saving the processor money and energy *(9, 19)*.

This study evaluates the shelf life of fresh crab meat packaged in traditional polyethylene snap-lid container and a new SimpleStep® tray sealed with a 10,000 cc/m^2/24 h OTR, Cryovac™ film. Identification of aerobic and anaerobic bacteria over the shelf life of the meat was also completed.

MATERIALS AND METHODS

The shelf life of fresh crab meat stored in two different package types (traditional polyethylene snap-lid container, and SimpleStep® tray sealed with a 10,000 cc/m^2/24 h OTR, Cryovac™ film) and incubated at 4°C was evaluated over a period of 12 days. Oxygen and CO_2 analyses were also conducted. The entire study was conducted in triplicate. The first two replications were performed with crabs harvested in Fall 2007; the third replication was performed with crabs harvested in Fall 2008.

Fresh crab meat sample preparation

Fresh, handpicked crab meat was obtained from a commercial processor in Cambridge, MD. The crab meat was purchased in the morning after picking was complete. On the day of purchase, 8 oz. of crab meat was transferred from several commercially packaged 12 oz. (340 g) snap-lid tubs into the polypropylene based SimpleStep® trays (8 oz., 227 g), and vacuum sealed with Cryovac™ 10,000 OTR film. Twenty-one commercially packaged snap-lid tubs and 21 SimpleStep® trays were packed in styrofoam ice chests with ice packs and shipped overnight to Blacksburg, Virginia. Upon arrival, the crab meat containers were stored at 4°C. Three SimpleStep® trays and three snap-lid tubs were evaluated for aerobic organisms on days 0, 4, 6, 8, 10, and 12. Three simple step trays and 3 snap lid tubs were evaluated for anaerobic organisms on days 0, 5, and 12. Testing day 0 was designated as the time the crab meat arrived at the Virginia Tech Food Science & Technology building.

At each sampling time, an 11 g sample of crab meat was aseptically removed from each container with a sterile spatula and placed in a separate sterile stomacher bag (Nasco, Ft. Atkinson, WI) with 99 ml of 0.1% peptone (Oxoid, Basingstoke, Hampshire, England). The samples were blended in a Stomacher Lab Blender 400 (Tekmar Co., Cincinnati, OH) for 30 seconds.

Enumeration of aerobes and anaerobes from fresh crab meat

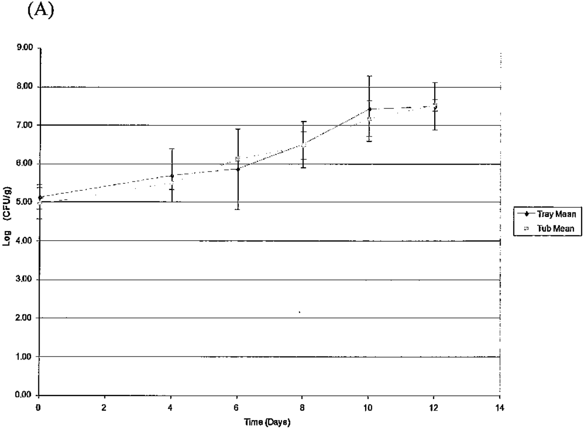
To enumerate aerobes from the crab meat, the homogenate was diluted using 9 ml peptone blanks, and dilutions were spread plated onto trypticase soy agar (TSA; BBL, Sparks, MD and MP Biomedicals, LLC, Solon, Ohio). Plates were incubated at 35°C for 48 h and colonies were counted.

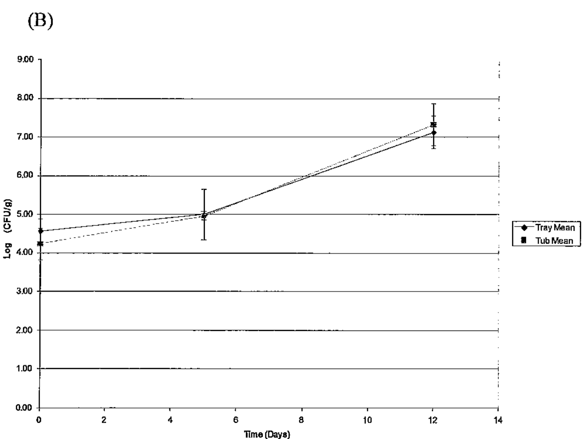
Anaerobic testing was performed according to methods outlined by Holdeman and Moore *(10)*. One ml aliquots from homogenate dilutions were placed in a glass anaerobe roll tube containing Brain Heart Infusion agar (BHI; BBL, Sparks, MD). After the tubes were inoculated, they were placed on a horizontal spinner (Bellco, Houston, TX) until the medium solidified. Roll tubes were incubated at 30°C for five days. After five days, the colonies were examined under a dissecting microscope and counted through the glass roll tube.

Cellular fatty acid identification preparation for aerobes

After colonies were counted, well-isolated colonies were picked and separately streaked onto TSA plates and

FIGURE 1. Aerobic (A) and anaerobic (B) plate count (log CFU/g) of microorganisms isolated from fresh crab meat packaged in SimpleStep® trays and traditional snap-lid tubs stored at 4°C for 12 days.





incubated for 24 h at 35°C. For mixed cultures, the microorganisms were repeatedly streaked until a pure culture was obtained. When a pure culture was obtained, the colonies were transferred into a clean (12 × 100), Teflon-lined screw capped tube, labeled and placed in a commercial freezer (-18°C, up to 15 days) until cellular fatty acid identification.

Cellular fatty acid identification preparation for anaerobes

After colonies were counted, well-isolated colonies were selected for identification. Under a constant stream of anaerobe grade CO_2, the colonies were pierced with a sterile needle and a sample of the colony was placed in a small anaerobic roll tube of cooked meat broth (CM, Difco, Sparks, MD) and placed in a 30°C incubator for 24 h. All cultures in the anaerobic CM were then grown on TSA plates for identification purposes, because results from preliminary studies demonstrated that no strict anaerobic microorganisms were present.

After 24 h, the broth tubes were checked and viewed for gas production and microbial growth. Under the constant stream of anaerobe grade CO_2 gas, a Pasteur pipette (FisherScientific, Pittsburg, PA) was used to dispense 6 drops of the cooked meat broth into a rubber stoppered 12 × 100 mm glass tube peptone-yeast extract basal medium broth (PYG), a custom-made solution *(10)*. The inoculated PYG solution was incubated for 24 h at 30°C. After 24 h, the PYG was centrifuged (Sorvall, GLC-1, Newtown, CT) at 3000 RPM for 10 minutes. The supernatant was removed and the remaining pellet subjected to cellular fatty acid identification.

Cellular fatty acid identification for aerobes and anaerobes

All aerobic and anaerobic identifications were performed using the Sherlock Microbial Identification System software (MIS, Microbial ID Inc., Newark, DE), which uses the cellular fatty acid profile to identify microorganisms. The procedure for cell sample preparation uses four reagents to saponify, esterify, extract and base wash the fatty acid extract, following MIS protocol *(12)*.

After base washing the fatty acid extract, approximately 100 µl of the washed extract was placed into 100 µl glass inserts (Agilent, Newark, DE). The individual glass inserts are housed in phenyl methyl silicone glass vials (25 mm × 0.2 mm ID × 0.3 µm film thickness) (Hewlett-Packard Co., Palo Alto, CA). Eleven mm crimp tops (Agilent, Newark, DE) were securely fastened to the top of the vials to prevent evaporation of the bacterial cellular fatty acid.

Standards and blanks were analyzed in the HP 5890A gas chromatograph (Hewlett-Packard Co., Palo Alto, CA) to standardize the equipment prior to the injection of the unknown samples. The chromatograph is equipped with a model HP 6763 autosampler (Hewlett-Packard), a flame ionization detector and a model HP-3392A integrator (Hewlett-Packard). The air gas flow rate through the chromatograph was 400 ml/min, 30 ml/min for hydrogen, and 30 ml/min for nitrogen. The temperature used was 250°C in the injection port and 300°C for the detector. After injection, the oven temperature of the apparatus was ramped from 170°C to 270°C at a rate of 5°C/min, followed by an additional increase from 270°C to 310°C at a rate of 30°C/min. This end temperature was held for 2 min before returning to 170°C prior to the injection of the subsequent sample.

The MIS software was used to calculate the percentage of area for each compound in its library, comparing it with the total area of the compound detected. Compounds were identified by using the Aerobic TSBA Version 4.0 Library and the 3.9 version for anaerobes.

Gas analyzer

The ratio of gas present in the SimpleStep® trays and the snap-lid tubs was analyzed using the CheckPoint O_2/CO_2 (PBI Dansensor America, Glenrock, NJ). Testing was conducted on days 0, 4, 6, 8, 10, 12, using 25 gauge 1 ½" sterile needles (Becton Dickinson, Franklin Lakes, NJ) and 13 mm filters (FisherScientific, Pittsburgh, PA). Tabs of weather stripping were placed on the Cryovac™ film and the snap-lid tops to protect the

TABLE 1. Bacteria isolated from fresh crab meat stored at 4°C in SimpleStep® trays with Cryovac™ 10,000 cc/m^2/24 h oxygen transmission rate (OTR) film and traditional polyethylene snap-lid tubs. Microbial colonies were evaluated at each sampling time. This list includes the collective microorganisms isolated from each package type throughout the study.

Microorganism	SimpleStep® trays	Polyethylene snap-lid tubs
Aerococcus viridans	+	+
Aeromonas caviae	+	
Acinetobacter calcoace		+
Actinetobacter johnsonii	+	+
Alcaligene	+	
Bacillus cereus	+	
Bacillus marinus	+	
Bacillus sphaericus		+
Carnobacterium piscicola	+	+
Cellulomonas fimi		+
Chromobacterium	+	
Corynebacterium ammoniage		+
Enterococcus faecalis	+	
Erwinia carotovora	+	
Exiguobacterium acetylicum	+	+
Kocuria varians	+	+
Kurthia gibsonii		+
Lactococcus plantarum		+
Micrococcus luteus		+
Myroides odoratus	+	+
Neisseria	+	
Pseudomonas nautica		+
Pseudomonas putida	+	
Shewanella putrefaciens	+	+
Staphylococcus arlettae		+
Staphylococcus caseolyticus		+
Staphylococcus chromogenes		+
Staphylococcus cohnii	+	+
Staphylococcus gallinarum	+	
Staphylococcus hominis		+
Staphylococcus kloosii	+	+
Staphylococcus sanguis		+
Staphylococcus warneri	+	
Staphylococcus xylosus	+	+
Streptococcus bovis	+	+
Streptococcus mutans	+	

+ indicates that bacterium was found

TABLE 2. Headspace gas composition (%) of SimpleStep® trays with Cryovac™ 10,000 cc/m²/24 h oxygen transmission rate (OTR) film and the traditional polyethylene snap-lid tubs for fresh crab meat stored at 4°C. (Packaging procedure for trial 3 was performed at a different location than trials 1 and 2.)

	Simple Step® trays with Cryovac film						Traditional polyethylene snap lids					
Days of Storage	Trial 1		Trial 2		Trial 3		Trial 1		Trial 2		Trial 3	
	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2
0	0.1	21.0	0.2	20.7	0.0	20.7	0.0	20.9	0.2	20.7	0.0	20.8
4	0.1	20.8	0.0	21.1	0.1	20.7	0.1	20.6	0.0	21.0	1.1	3.9
6	0.1	20.9	0.0	20.8	0.1	20.6	0.1	20.9	0.0	20.7	8.6	0.8
8	0.1	20.9	0.0	20.9	0.0	20.8	0.1	20.9	0.0	21.1	12.5	2.7
10	0.1	21.1	0.0	20.7	0.1	20.6	0.1	0.9	0.0	20.6	12.8	0.5
12	0.1	20.8	0.0	20.7	0.1	20.6	1.1	20.8	0.0	20.8	0.1	16.5

integrity of the package prior to the insertion of the needle.

Statistical analysis

Data were analyzed by use of a completely randomized design. All statistical analyses were conducted using SAS, version 9.1 (SAS Institute, Cary, NC). The mean log survival of aerobic and anaerobic bacterial growth from standard plate counts on TSA and BHI agar (respectively) were analyzed using the general linear model (GLM), and a model mean of the data was compared with the method of least squares means (LSD) for effect. The data readings from the O_2 and CO_2 gas analysis output and coliform MPN were also analyzed using the GLM, and the model means of the data were compared using LSD.

RESULTS

Microbial spoilage in the shelf-life study

For this study, spoilage was defined as microbial counts at or above 7.0 log CFU/g *(14)*. The aerobic plate count of the crab meat on day 0 for the SimpleStep® trays was 5.12 log CFU/g (±0.32) and 4.97 log CFU/g (±0.40) for the traditional snap-lids, respectively. By 10 days of storage, the aerobic counts of the fresh crab meat reached 7.0 log CFU/g (considered spoiled) in both package types (Fig. 1 A). By day 12, aerobic plate count for the SimpleStep® trays was 7.50 log CFU/g (±0.62) and 7.53 log CFU/g (±0.15) for the traditional snap-lids, respectively. There was no significant difference in the aerobic counts between package types on each sampling day ($P > 0.05$).

The anaerobic microbial counts on day 0 for the SimpleStep® trays was 4.57 log CFU/g (±0.32) and 4.23 log CFU/g (±0.40) for traditional snap-lids, respectively. The anaerobic plate count on day 12 for the SimpleStep® trays was 7.13 log CFU/g (±0.42) and 7.33 log CFU/g (±0.55) for the traditional snap-lids. Anaerobic plate counts after the twelve day incubation period showed that anaerobic growth between the two package types was not statistically significant ($P > 0.05$). An evaluation of the bacterial organisms identified in the SimpleStep® trays and polyethylene snap-lid tubs is shown in Table 1.

Gas analysis

The concentration of O_2 and CO_2 gas remained consistent in both types of packaging during the first 8 days of sampling in the first two trials. On day 10 for trial 1, the concentration of CO_2 in the polyethylene snap-lid tubs had increased slightly, and O_2 levels had decreased. By day 12, the CO_2 was still slightly higher in the snap-lid tubs, but O_2 increased to normal levels. Both CO_2 and O_2 remained constant throughout the second repetition. In the third repetition, CO_2 increased on day 6 and remained elevated until day 12. The O_2 levels dropped on days 4–10, but had recovered on day 12. There were differences in O_2 levels within replications ($P < 0.05$). Carbon dioxide concentrations were higher in the snap-lid containers than in the SimpleStep® trays ($P < 0.05$). Overall, the gas concentrations were not significantly different between sample days ($P > 0.05$) (Table 2).

DISCUSSION

There were no differences in shelf life of crab meat packaged in traditional polyethylene snap-lid cups versus the SimpleStep® trays with Cryovac™ 10,000 OTR film ($P > 0.05$). The crab meat in the SimpleStep® tray with the Cryovac™ 10,000 OTR film showed aerobic bacterial growth similar to that of the meat in the polyethylene snap-lid cups. Gates et al. tested oxygen barrier pouch packaging, non-barrier pouches and vacuum-skin packaging on fresh crab meat and concluded that no packaging material improved the microbiological shelf life *(9)*. The results from this study support Gates et al., indicating that there is no difference in shelf life of the crab meat

in either of the package types that were tested *(9)*.

Environmental conditions (e.g., picking and packing room temperature, Chesapeake Bay water temperature etc.) and handling practices (e.g., picking table cleanliness, use of bare hands or clean gloved hands, etc.) at the crab meat processor and the purchaser's establishment all contribute to the level of contaminants in the fresh crab meat and thereby affect the data. Spoiled meat was determined as meat with bacterial counts at or above 10^7 CFU/g *(14)*. No strict anaerobes were detected in any of the replications, which is similar to the results from a fresh crab meat study performed by Suklim et al. *(20)*. Additionally, Ward et al. noted that when anaerobic colonies were examined, the organisms isolated anaerobically were identified as facultative lactobacilli *(22)*, further indicating that no strict anaerobes were present in the sampled crab meat. Both packaging types were found to have a variety of *Staphylococcus* species, likely a result of the handling conditions. Also isolated were different spoilage bacteria, including *Schewanella*, *Carnobacterium*, and *Pseudomonas* species. *Pseudomonas* species have been previously reported as major spoilage organisms in seafood *(22)*. The processor from whom the crab meat was purchased advertises Chesapeake Blue Crab meat and Indonesian pasteurized crab meat. Interestingly, *Chromobacterium* spp. was isolated from the fresh crab meat purchased from this company. *Chromobacterium* spp. is a component of the normal flora of water and soil of tropical and subtropical regions of the world, suggesting that the domestically picked crab meat was contaminated with microorganisms *(17)* from the imported crab meat.

Before deciding if a new packaging material should be used, it is necessary to know what will cause product deterioration and the effects of commercial shipping and handling on package failure rate. Ideally, the expectation of new packaging through advanced technologies is to extend the shelf life of perishable food products. The results of this microbial shelf-life study suggest that there were no differences in microbial concentrations between the SimpleStep® trays with Cryovac™ 10,000 OTR film or the polyethylene snap-lid cups that can be attributed to package type ($P >$ 0.05).

Regardless of package type, if CO_2 is allowed to build up, the crab meat can deteriorate quickly. The difference in the concentration of CO_2 in the SimpleStep® trays compared with the polyethylene snap-lid tubs on days 10 and 12 (first repetition) and day 4 through 12 of the third replication may be due to the production of CO_2 gas from fermented lactose or the consumption of O_2 by aerobic microorganisms *(13, 16)*. Carbon dioxide can inhibit the growth of spoilage microorganisms, increasing the shelf life of certain food products *(1, 3)*. No consistent trends in CO_2 levels were observed in any repetition, making it difficult to identify a cause for the CO_2 gas fluctuation. Both CO_2 increases in replications one and three occurred in the polyethylene snap-lid tubs, suggesting that the tubs may be less efficient in releasing CO_2 into the outside environment compared with the Cryovac™ 10,000 cc/m^2/24 h OTR film.

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