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Evaluation of Decay and Microbial Growth in the *Ictalarus punctatus* (Channel Catfish)

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ABSTRACT

The microbial changes and decay in the channel catfish (*Ictalarus punctatus)* were investigated. Fish fillets stored in low temperatures and fish fillets stored in low temperatures at low oxygen levels were found to begin decaying near two days of storage. Low temperatures were found to inhibit the growth of microbial colonies up to about ten days on the fillet only one day old and low temperatures inhibited microbial growth for three days on the fish fillet that had been stored for five days. Low temperature and low oxygen environments found to inhibit growth of microbial colonies up to two to three days on the fish fillets stored for only one or two days and it inhibited microbial growth for only one day on the fish fillet stored for five days.

Keywords: *Decay, Shelf life Ictalarus punctatus, chilling, Low oxygen*

INTRODUCTION

The most important ways used to preserve fish in both tropical and temperate climates is by chilling to about 0^{o} C (Surti et al, 2001). Geevarethnam and his colleagues (2008) researched the microbial and biochemical quality of grouper fish (*Epinephelus chlorostigma*) stored in dry ice and water ice. They state that the rate of deterioration in fish is mainly dependent on temperature, and the use of low storage temperature can inhibit deterioration and decay (Geevarethnam et al, 2008). Surti and his colleagues studied the effects of storage at tropical ambient temperatures on the quality and shelf life of grouper (*Plectropomus maculates*). They found that spoilage of fresh fish can be caused by a number of inter-related systems (Surti et al, 2001). Some factors found to cause spoilage include degradation of nucleotides with the successive formation of hypoxanthine, formation of trimethylamine (TMA), development of oxidative rancidity and the action of certain bacteria (Surti et al, 2001).

A method that helps slow the deterioration process of seafood is chilling. Geevarethnam and his colleagues state the atmospheric surroundings of the seafood can also have an affect on the shelf life of a fish fillet. If the surrounding atmosphere is modified to reduce the concentration of oxygen, the product's shelf life is increased, and therefore would have a decreased rate of deterioration and decay. The shelf life is increased because of the reduction in the rates of chemical oxidation by oxygen and of the growth of aerobic microorganism (Geevarethnam et al, 2008). When transporting fish, crushed ice is usually used at a 1:1 ratio of ice to fish. Some transporters use a mix of ice and water to store the product during transportation. This can cause textural toughness, nutrient loss, and protein extractability (Geevarethnam et al, 2008).

Carbon dioxide is the most important gas used in modified atmospheric packing of fish (Geevarethnam et al, 2008). Carbon dioxide is so important in packing because of its bacteriostatic and fungistatic characteristics. Because of these characteristics, carbon dioxide rich atmospheres have become very popular in distributing food.

Another method of transporting seafood and keeping it chilled is using dry ice. Dry ice acts as a coolant when transporting seafood and acts as insulation enveloping the fish upon evaporation (Geevarethnam et al, 2008). Dry ice acts primarily to increase the carbon dioxide and to retard the spoilage of the fish fillets. Dry ice can be mixed with water in order to save on shipping weight, to be more cost efficient, and to extend the cooling energy of water ice (Geevarethnam et al, 2008). Geevarethnam and his colleagues found in their laboratory that dry ice combined with water was an efficient method of keeping fish fresh and create a longer shelf life. The combination of dry ice and water was in a ratio of 20:50 weight for weight.

Marroquin and his colleagues explain in their article that the shelf life of channel catfish largely depends on post harvest handling and storage conditions (Marroquin et al, 2004). Post handling techniques include hand dressing or mechanical dressing (Marroquin et al, 2004). In both techniques, fish fillets are immediately chilled (Marroquin et al, 2004).

My project will evaluate the decay and microbial growth of the *Ictalarus punctatus* using the techniques performed by Geevarthnam and his colleagues (2008).

MATERIALS AND METHODS

In the experiment by Geevarthnam and his colleagues (2008), they purchased their samples from a nearby fish-landing center. I purchased my fish fillets from McPherson's local Dillon's grocery

store. My sample included one *Ictalarus punctatus* fillet. Dillon's fish fillets are prepared, beheaded and ready to cook. The fish sample was iced by directly mixing with crushed ice. I brought my fish sample back to the lab and performed my next steps.

My storage methods copy those used by our local Dillon's Grocery Store. When fish first reach the Dillon's grocery store they are stored in a cooler at 0^0 C. Once fish are beheaded they are stored in a case with ice at -1 to 2^0 C. Dillon's keeps their fish on shelf and available for purchase for two to three days.

Fish muscle was cut into small pieces that weighed about half a gram (Geevarthnam et al, 2008). Next, five grams of the sample were homogenized using 45 milliliters of sterile trypticase soy broth (Geevarthnam et al, 2008). Dilutions start with a ten milliliter sample of the fish fillet mixture taken directly from the sterile blender and placed in a test tube. Next I made serial dilutions of 10^{-1} in sterile trypticase soy broth. I carried my dilutions out to 10^{-4}. The mixture was appropriately diluted and plated onto the trypticase soy agar.

Solutions were plated on trypticase soy agar every day for five to six days. I tested the microbial growth of fish stored at chilled temperatures as well as fish chilled and stored at low oxygen levels. I used an anaerobic container to store the fish at low oxygen levels. By lowering the oxygen levels I was be able to determine how much it affects the rate of deterioration compared to just chilling the fish. There are four variables in my experiment requiring eight sets of plates. One set of plates measures the microbial growth of the *Ictalarus punctatus* when stored at five degrees Celsius and the other set of plates measures the microbial growth of the *Ictalarus punctatus* when stored in an anaerobic container at 37^o C. Each set had two plates made from the fish sample stored on ice at low temperatures and two plates made from the fish fillet stored on ice at low temperature, in a low oxygen atmosphere. Each of these plates included plates made with the fish samples that have been diluted to 10^{-3} and 10^{-4}. Plates were inoculated with one milliliter of diluted solution.

Next, I sterilized the spread plate tool by dipping it in alcohol and burning it off. Using this tool I was able to spread my solution evenly over my agar. After plates were prepared they were placed in their correct incubators. The plates measuring the low temperature variable were placed in the five degree Celsius incubator and the plates measuring the low oxygen variable were placed in an anaerobic container and stored in the 45^0C incubator. The Anaerobe container system BBL was set up to imitate a low oxygen environment. Plates were stacked on top of each other and placed in the jar. Gas Pak EZ packets, Ref # 260678, were then added to the jar and it was sealed tight. Plates were left in incubators and checked every twenty four hours for microbial growth.

In my second run of data I changed my variables from two variables to four variables. The next data collection the fish were stored partly on ice in the refrigerator and the other half on ice in a low oxygen environment in the refrigerator. This yielded eight plates per day instead of four.

During the second run of data, decreased amounts of sample were used and spread plated onto trypticase soy agar. In the second data run, 0.5 mL of solution were used instead of 1 mL. After serial dilutions were made 0.5 mL of solution was pipetted onto trypticase soy agar. Plates were incubated at low temperatures in the 5^o C incubator and at low temperatures in an anaerobic container in the 37^o C incubator. This procedure was repeated every day for five days.

In the third data run the variables of the experiment changed. Instead of just storing the fish fillet at low temperatures I cut the fillet in half and stored half at just low temperature and the other half at low temperature in a low oxygen environment. Two variable yields eight plates total each day, for a total of 40 plates over a five day period.

I followed the same step as the first two data runs on the third run. After homogenizing a 5g sample of fish in 45 mL of trypticase soy agar and performing the appropriate dilutions, 0.5 mL were spread plated onto trypticase soy agar. These steps were repeated for the fillet stored at low temperatures and for the fillet stored at low temperatures in a low oxygen atmosphere. For each fillet samples were made and plated at 10^{-3} and 10^{-4}.

Once colonies started to grow I counted them and recorded the number of colonies daily. Next I compared the microbial growth of the plates to determine the rate of deterioration of the *Ictalarus punctatus*.

RESULTS

Freshness is essential for the quality of the final product in many kinds of products (Karoui et al, 2007). Ways of extending the quality and freshness of fish included chilling, freezing, and storing in low oxygen environments. My experiment has compared the method of storage of fish fillet at low temperatures versus storage of fish fillets at low temperatures and in a low oxygen environment. By plating samples from these two variables I have obtained bacterial counts over a period of seven days.

The first trial run after 24 hours of incubation found that no colonies had grown on the low temperature plates and only a few colonies had grown on the low oxygen plates. After 48 hours of incubation still no colonies had grown on the low temperature plates. After 72 hours of incubation there were still no colonies on the low temperature plates, but colonies had started to appear on the low oxygen plates as

shown in Table 1.

After 96 hours of incubation more growth is apparent on both sets of plates, and on day six, after 120 hours of incubation we see the most growth taking place.

Table 1 shows the bacterial count for the first data run. In the first run I found the need to continue out my data longer to find where the number of colonies plateaus.

In the second data run after 24 hours of incubation no growth had occurred on the low temperature plates and only a few colonies grew on the low oxygen plates. After 48 hours of incubation no growth had occurred on the low temperature plates and just a few colonies grew on the low oxygen plates. After 72 hours still no growth on any of the low temperatures plates had occurred but growth on the low oxygen plates had increased.

After 96 hours, growth appears on the low temperature plates from day one and increased growth continues on the low oxygen plates. After 120 hours of incubation, the low temperature plates start to show more growth on all the plates and the low oxygen plates show lots of growth.

I performed the same procedure as the first two data runs as in my third run but only plated 0.5 mL sample onto the trypticase soy agar.

Plates made on day one for the fish fillet stored at low temperature did not yield any growth until about ten days of incubation. These plates did not have much growth total after thirteen days of incubation. The plates stored in the anaerobic container yielded microbial growth much faster. Colonies appeared on those plates after only 24 hours of incubation.

Plates made on day two stored at low temperatures did not produce colonies until five days of incubation had occurred. The plates of day two that were stored in a low oxygen environment produced colonies after two days of incubation.

Plates made on day three stored at low temperatures produced colonies after four days of incubation and the plates stored at low oxygen produced colonies after two days of incubation. The plates stored in low oxygen were completely covered by microbial growth and uncountable after five days.

Plates made on day four stored at low temperatures produced colonies after three days of incubation and plates stored at low oxygen produced colonies after only 24 hours of incubation. Microbial growth occurred rapidly on these plates and completely covered the plates after four days of incubation.

Day five plates stored at low temperatures produced colonies after four days of incubation and plates stored at low oxygen produced colonies after 24 hours of incubation. Both sets of plates made for the low temperature and low oxygen, were completely covered by bacteria after six days of incubation.

Further data analysis were carried out on data from run three and P values were calculated along with the log10 of the final amount of colonies grown for each plate.

As Table 1 shows the first column is the final number of colonies found in each day. The second column calculates the log10 of each number. The third column displays a few more important values calculated with the data. The P value was found to be less then 0.05, therefore the regression is significant. With the data found I was able to determine the formula for y=mx+b. This formula is as follows, y=0.520(x) + -0.357. The R square value is a statistical measurement of how well a regression line approximates real data points. The closer the R square value is to one, the greater the ability to predict a trend. The R square value was found to be 0.8915 in data Table 1, which shows a strong trend.

1	0	Sig F= 0.0157020486351652
10	1	B= -0.357462977084364
8	0.90308999	m= 0.520099952194873
105	2.0211893	R Square Value= 0.891458320692614
123	2.08990511	

Table 1. Fish fillets stored on ice in refrigerator and plates stored at low temperatures in 5^{o}C incubator.

In Table 2 the columns represent the same figures as in column A. Number of colonies found recorded first followed by their log10 values. The P value for this data was found to be 0.0157, which is less then 0.05, therefore shows the regression to be significant. The formula for this data is as follows, y=0.392(x) + 0.173. The R squared value was found to be 0.794, which is still pretty close to one and therefore shows a strong trend.

Low Temp Plates. Fish fillets on Ice/O2		
3	0.47712125	Sig F= 0.0425211234508884
10	1	B= 0.172965338967318
50	1.69897	m= 0.391602207149041
18	1.25527251	R Squared Value= 0.793771705187954
203	2.30749604	

Table 2. Fish fillets stored on ice in a low oxygen atmosphere and plates stored at low temperatures in 5^{o}C incubator.

In Table 3, the P value is 0.640, therefore the regression is not significant. The formula is y=0.0522(x) + 1.784. The R squared value is 0.082.

Low Oxygen Plates. Fish fillets on Ice		
142	2.15228834	Sig F= 0.64037827993137
30	1.47712125	B= 1.78396329187381
70	1.84509804	m= 0.0521684323682342
142	2.15228834	R Squared Value= 0.08203165998379
119	2.07554696	

Table 3. Fish fillets on ice in refrigerator and plates stored in anaerobic container in 45^{0}C incubator.

In Table 4, the P value is 0.399, therefore the regression is not significant. The formula for this data is y= 0.283 (x) + 0.750. The R value is 0.243.

Low O2 Plates. Fish fillets on Ice/O2		
64	1.80617997	Sig F= 0.398675090605863
1	0	B= 0.749514801376245
89	1.94939001	m= 0.281633663462278
101	2.00432137	R Squared Value= 0.243105641484134
163	2.2121876	

Table 4. Fish fillets on ice in refrigerator in a low oxygen atmosphere and plates stored in anaerobic container in 45^{0}C incubator.

As Figure 1 illustrates all the data had a similar slope and rate of decay. By day three it is visible that most of the data points are in the top half of the graph indicating that the microbial growth is at its highest.

DISCUSSION

During my evaluation of fish decay and microbial growth I was able to assess the amount of bacterial growth that occurred on the fish fillets by plating a solution onto trypticase soy agar and counting the bacterial growth on the plates.

Three data runs were completed in order to assess the bacterial growth of the *Ictalarus punctatus.*

During the first run the amount of solution used was too high and caused microbial growth on the plates to occur to rapidly too count colonies. In subsequent runs I reduced the amount of solution used from 1 mL to 0.5 mL. This was successful and yielded a sufficient amount of colonies to analyze.

In the first two data runs for my project I only had two variables, two plates grown at low temperatures and two plates grown in a low oxygen environment. Each variable had plates at 10^{-3} and 10^{-4} concentrations which yielded four plates. For the third data run the amount of variables was changed from two to four. Plates stored at low temperatures

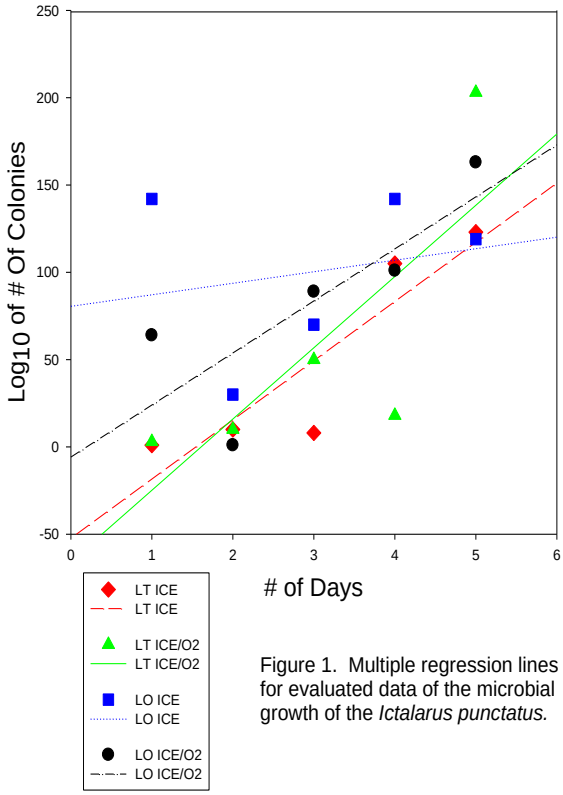

Figure 1. Multiple regression lines for evaluated data of the microbial growth of the *Ictalarus punctatus.*

and plates stored in a low oxygen environment each had plates made from fillets stored differently. Part of the fillet was stored at low temperatures in the refrigerator and the other half was stored at low temperatures in an anaerobic container. Each variable had plates at 10^{-3} and 10^{-4} concentrations which yielded eight plates each day.

In the experiment by Geevarthnam and colleagues, grouper fish stored 100% water ice yielded bacterial counts after only one hour of storage and continued to increase with the hours.

Some recommendations for changes in my project would include checking for bacterial growth more often then every 24 hours such as Geevarthaman and his colleagues did. This might help get a more accurate count on the colonies.

The next steps for research would be to find more efficient ways to help inhibit microbial growth of fish fillets and therefore help reduce waste.

In conclusion, microbial growth was measured and found to result in decay of the *Ictalarus punctatus.* Low oxygen environments did not seem to inhibit growth of colonies any more then the low temperature storage methods.

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