Final Report

Effect of High Pressure Treatment on Omega-3 Fatty Acids in Fish Muscle

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ABSTRACT

Cardiovascular health benefits associated with omega-3 fatty acids have been well documented. Seafood is recognized as an excellent source of omega-3 fatty acids in the diet. Consumer's prize and purchase seafood as either fresh or "fresh frozen." Unfortunately, seafood is highly perishable with a 14 day shelf-life and techniques to extend shelf-life beyond frozen processing cause quality changes consumer's will not accept. High pressure processing (HPP) may extend the shelf-life of seafood while maintaining the fresh-like characteristics consumers demand. However, HPP may promote the oxidation of omega-3 fatty acids, thereby loosing seafood's nutritional and market advantage. This research was to establish the effect of high-pressure conditions on fatty acid profile, to evaluate high-pressure on fish lipid from freshwater and salt-water fish species, tostudy high-pressure treatment on a purified fish lipid system, and toinvestigate high-pressure treatment on the activities of endogenous muscle pro- and antioxidants. HPP reduced bacterial numbers and improved shelf-life of seafood. Reductions of 3-6 logs were achieved with HPP and shelf-life could be extended. Lipid oxidation increased as pressure increased especially during storage over 3-6 days for all species tested. After HPP, fillets appeared as if they were cooked; although temperature during processing never went above 35^{o}C. This cooked appearance affected the surface color of the fillet with L-value and b-value increasing with pressure while a-value decreased. Overall, HPP shows promise in extending the shelf-life of seafood; however, further research is needed to control and understand lipid oxidation and color/appearance changes resulting from this process.

EXECUTIVE SUMMARY

Omega-3 fatty acids are polyunsaturated fatty acids required in the diet. Intake of these, specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been shown to help reduce the risk of cardiovascular disease. Seafood can be an excellent source of EPA and DHA, although extremely variable depending on fish species. Good sources of omega-3 fatty acids include all types of salmon and tuna, sardines, herring, mackerel, black bass, bluefish, carp, pompano and channel catfish. Although recognized for its health benefits, seafood consumption has remained relatively constant with close to 70% consumption as fresh or "fresh frozen."

Seafood is highly perishable with a 14 day shelf-life for a fresh or thawed product. Usually beyond 7 days the product is considered a lower grade and frequently sold at reduced cost or discarded. Processing techniques that can extend the shelf-life of seafood past 14 days dramatically change the sensory attributes and characteristics of the product beyond the fresh quality demanded by consumers. High pressure processing (HPP) has been applied to foods as a preservation method with its major advantage being that it maintains the fresh quality attributes of food. One problem with HPP is that it can affect proteins and cell membrane function. Lipid oxidation of seafood is well known and many researchers have demonstrated that various proteins from seafood are involved as catalysts. However, very little information exists on HPP of seafood, and more specifically, its effect on oxidation of omega-3 fatty acids.

Thus, the objectives of this work are 1) to establish the effect of high-pressure conditions (time, pressure, temperature) on fatty acid profile of the fish lipid fraction; 2) to determine the effect of high-pressure on fish lipid from freshwater and salt-water fish species; 3) to study the effect of high-pressure treatment on a purified fish lipid system; and 4) to investigate the effect of high-pressure treatment on the activities of endogenous muscle pro- and antioxidants.

Fish studied included mahi mahi, salmon, rainbow trout and tilapia. The HPP was done in a Stansted laboratory scale unit at pressures ranging from 150 – 600 MPa. Lipid oxidation was measured by analyzing secondary products of oxidation by the thiobarbituric acid reactive substance (TBARS) method. Total aerobic microbial growth before and after HPP treatment was determined using PetrifilmTM according to the official AOAC method. Color was measured throughout storage by the Color Machine Vision System (CMVS) consisting of a light box and a CCD color camera connected to a computer with a video frame grabber. The software developed was used to capture images, and to obtain color results based on RGB and L, a*, b* values.

HPP reduced bacterial numbers and improved shelf-life of seafood. Reductions of 3-6 logs were achieved with HPP and shelf-life could be extended. Lipid oxidation increased as pressure increased especially during storage over 3-6 days for all species tested. After HPP, fillets appeared as if they were cooked; although temperature during processing never went above 35^{o}C. This cooked appearance affected the surface color of the fillet with L-value and b-value increasing with pressure while a-value decreased. Overall, HPP shows promise in extending the shelf-life of seafood; however, further research is needed to control and understand lipid oxidation and color/appearance changes resulting from this process.

PURPOSE

Statement of Problem: The health benefits associated with the consumption of omega-3 fatty acids from seafoods is one of the most promising developments in nutrition research in the past 20 years. As a result, seafood remains a healthy, attractive choice to consumers. The consumption of seafood in the U.S. has remained constant since 1996 at 14.9 pounds per person and approximately 68% of this is consumed as fresh or frozen fish and shellfish.

Omega-3's, are polyunsaturated fatty acids, which are also found in certain plants such as flaxseed, pumpkin seed, and walnuts. They are essential fatty acids that the human body cannot create without first obtaining them from food. Linolenic acid, the primary omega-3 fatty acid, can be obtained through many fats, oils, nuts, and soybeans. However, while eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) - which are also from the omega-3 family - can both be created by the body in the presence of linolenic acid, they are bestavailable through breast milk for infants and seafood for adults.

Research from epidemiological studies in different populations,› clinical trials in patients and healthy subjects, animal experiments, biochemical studies, and cell culture experiments continues to expand our understanding of how these substances contribute to health. While there is still considerable research and argument in answering such basic questions as, how much do consumers require to see beneficial health effects, there have been gains in understanding how omega-3 fatty acids function to promote health. Several health benefits have been associated with the regular consumption of omega-3 fatty acids. Two of these –heart health and prenatal development- are especially germane to consumer's interest because of the large number of people potentially affected.

The total amount of fat in fish is extraordinarily variable. It ranges from 0.2 to 23.7%. Indeed, fish can be classified by their fat content into four categories: Lean (<2% fat), low (2-4% fat), medium (4-8% fat) and high (8% fat>). High oil fish which are good sources of omega-3 fatty acids include all types of salmon and tuna, sardines, herring, mackerel, black bass, bluefish, carp, pompano and channel catfish. Because of the polyunsaturated nature of fish lipids, they may undergo different reactions. In fatty fish species, lipid oxidation represents a serious problem. These fish contain more free lipids and more dark muscle in which oxidation takes place more rapidly than in white muscle.

Significant lipid degradation in fish muscle during refrigerated and frozen storage is due to enzymatic hydrolysis and leads to both a decrease in phospholipids and an increase in free fatty acids.› Free fatty acids are regarded as more susceptible to autoxidation and usually cause subsequent oxidative rancidity of fish. Moreover, textural changes in fish muscle have been reported to be caused by the increase in free fatty acid content. Restriction of the accumulation of free fatty acids could, therefore, be effective in preserving the quality of fish during storage. One of the effective means of reducing the hydrolytic degradation of fish muscle lipids is heat processing, leading to inactivation of the corresponding lipolytic enzymes. However, other components including vitamins, and the polyunsaturated fatty acids themselves, are sufficiently labile that autoxidation could be accelerated by cooking. Recently, treatment of food and foodstuffs with high hydrostatic pressures (HHP) has been tentatively applied to food processing and preservation.The engineering aspects of HHP applications in the context of food processing, safety and quality, and the effect of HHP on ice-water transitions have been recently discussed.› One of the distinct advantages claimed for pressurization in food processing and preservation is that heat-labile compounds undergo limited degradation compared with heat processing.The response of food products to HHP processing is complex, affected by processing parameters, and product characteristics, such as applied pressure, duration of compression, temperature, product pH and water activity. The literature about the effect of high pressure on lipids is indeed sparse, and even more so, is its effect on fish lipids.

Oshima et al. used pressure levels between 200 to 610 MPa for 15 to 30 min and suggested that isolated extracted marine lipids were more stable against autoxidation than lipids present in intact muscle. Angsupanish & Ledward observed little changes in thiobarbituric number (TBA), an index of lipid oxidation, on cod muscle pressurized at 200 MPa for 20 min. However, in the same experiment, TBA values increased when samples were treated at 400 MPa or higher for 20 min and continued to increase during the 7 days of storage. Recently Sequeira-Munozsuggested that HHP treatment of fish fillets prior to frozen storage may have a curtailing effect on the oxidation of lipids in carp fillets. High pressure could significantly affect the following properties for lipid oxidation:

- May increase the catalytic role pro- and antioxidants play in fish muscle

- Can denature proteins, such as hemoglobin and myoglobin, which could lead to an increased ability to oxidize lipids
- May inactivate fish lipoxygenases, which would reduce lipid oxidation
- Can influence the integrity of cellular membranes, which could not only increase the susceptibility of the membrane lipids to undergo oxidation, but may also lead to the decompartmentalization of soluble pro- and antioxidant compounds in cellular and sub-cellular structures

As very limited information exists on the effect of high pressure on the oxidative stability and integrity of fish lipids, it is important to obtain information on how lipids behave during high pressure seafood processing. Thus, this research is intended to provide a better understanding of the effect of high pressure on fish lipids and its degradation and to use this information obtained to rationalize more effective strategies for preserving the premium quality of the "fresh" fish during high pressure processing.

Objectives: i) to establish the effect of high-pressure conditions (time, pressure, temperature) on fatty acid profile of the fish lipid fraction; ii) to determine the effect of high-pressure on fish lipid from freshwater and salt-water fish species. iii) study the effect of high-pressure treatment on a purified fish lipid system; and iv) Investigate the effect of high-pressure treatment on the activities of endogenous muscle pro- and antioxidants.

APPROACH

Fish Samples: Both mahi mahi and salmon were purchased from Save-on-Fish Company (Tampa, FL). Rainbow trout were purchased from a local market and tilapia was supplied by Dr. H. Kristinsson.

High Pressure Processing: The high-pressure equipment consisted of a Stansted laboratory scale unit(Stansted Fluid Power, Stansted, Essex, UK) with a pressurization chamber of 114mmdiameter and 243mm height, providing a usable volume of approximately 2,480 ml. Skinned fillets from fresh fish were vacuum packaged and treated at different pressures (from 150 to 600 MPa) for 15 min initially at room temperature. Some samples were then stored for 6 days at 4°C. The red muscle was analyzed every two days for lipid oxidation by measuring thiobarbituric reactive substances (TBARS). Total aerobic count and color analysis were also performed every two days on the whole muscle. Images of the fillets were captured using a color machine vision system (CMSV) equipped with a video camera and average color parameters (L*, a* and b* values) were determined.

Lipid Oxidation Methods: Development of lipid oxidation in fresh-water and salt-water fish species was measured by analyzing secondary products of oxidation in the red muscle according to the thiobarbituric acid reactive substance (TBARS) method. Dark muscle tissue (5 g) was blended with 15 ml of TCA extracting solution (7.5% trichloroacetic acid in water, 0.1% propyl gallate and 0.1% EDTA) for 30 s in a plastic beaker, the suspension filtered using a Whatman #1 filter paper and then 2 ml of suspension was mixed with 2 ml of TBA (thiobarbituric acid) in a screwed cap tube. The tube was vortexed for 10 s, and placed into boiling water for 40 min. Finally, the tube was placed into ice for 5 min, and optical density of samples was measured at 530 nm.

Microbiological analysis: Total aerobic microbial growth before and after HPP treatment was determined using PetrifilmTM (3M Laboratories, St. Paul MN) according to the official AOAC method. The 3M petrifilm aerobic plate is a ready made medium that contains standard nutrients, a cold water soluble gelling agent and a tetrazolium indicator dye which facilitates colony enumeration. Analysis was done on 10g fish muscle mixed with 90 ml sterile pre-filled dilution vials of phosphate buffer solution at pH 7.2 (Hardy Diagnostic, Santa Maria CA). The solution was then blended in a stomacher for 1 min and the pH adjusted to 6.6-7.2 with 5 N NaOH and then serially diluted (10^{-1}-10^{-7}). For inoculation, 3M Petrifilm TM was placed on a sterile flat surface and 1.0 ml of the sample was placed at the center of the film and spread by a sterile plastic spreader to an area of ~20 cm^2. Duplicate inoculations were conducted for each dilution and no more than 10 plates were stacked at 35.5°C for an incubation time of 48±3 hours.

Color Analysis: Color was measured throughout storage by the Color Machine Vision System (CMVS) consisting of a light box and a CCD color camera connected to a computer with a video frame grabber. The software developed was used to capture images, and to obtain color results based on RGB and L, a*, b* values. Fish fillets were placed in the light box and a digital camera captured a picture of fish fillets for each analysis time point. L*a*b* values were calculated by using color analysis program.

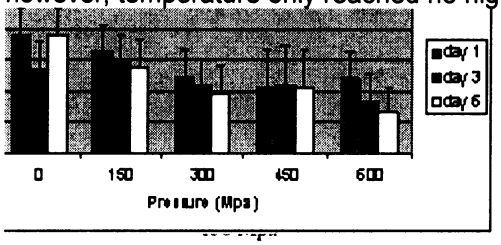
Project management: All work was performed in the facilitiesat the University of Florida, Food Science and Human Nutrition Department, Gainesville, FL.

- Dr. M.R. Marshall was responsible for all phases of the project and in assisting with the chemical analysis of the samples.
- Dr. H.G Kristinsson was in charge of sample preparation for HHP treatment and worked closely with the student performing chemical analysis and monitoring during storage.
- Dr. M.O. Balaban worked with the student doing the high pressure processing of the seafood samples.
- Mr. Y. Yagiz is a master's student who performed all of the processing runs and chemical analyses for the project.

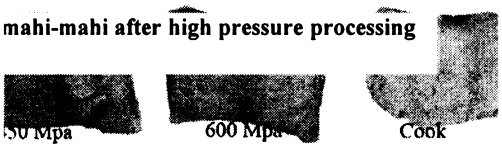
FINDINGS

High Pressure Processing of Fish: High-pressure processing (HPP) on rainbow trout, mahi-mahi and tilapia was performed. It was decided to examine HPP's influence on whole fish before going into the model and molecular systems. Figure 1 shows the profiles for pressure, temperature and time at various

operating processing pressures for mahi-mahi and tilapia. For 250 and 400 MPa, the time to reach operating pressure was relatively short within 5 min. Temperature increased to no more than 30°C. However for 550 MPa, note that the time to get to operating pressure was close to 2 hr. This was caused by trying to maintain the temperature at between 15-20°C, which caused the ramp effect as seen with pressure. The unit in maintaining temperature will stop increasing pressure until the temperature range is acquired and then pressure is increased again. This is why it took over two hours to reach operating pressure. It was felt that increasing temperature quickly to operating pressure would not hinder further studies and thus temperature was not controlled. Figure 2 shows the pressure and temperature profile for rainbow trout at pressures from 150 to 450 MPa. The profiles are very similar at the lower pressures for mahi-mahi and tilapia, although as pressure increased, time to reach operating pressure also increased. The high pressure at 600 MPa treatment took over 10 min to reach operating pressure, however, temperature only reached no higher than 35°C.



mahi-mahi after high pressure processing



high pressure processing and storage.

e 16. Photograph of the surface of salmon after high
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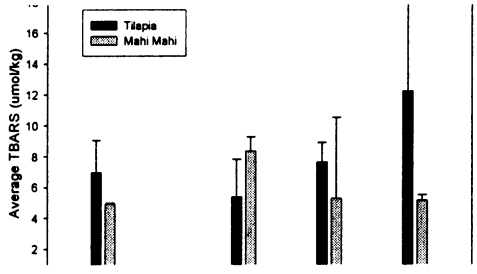
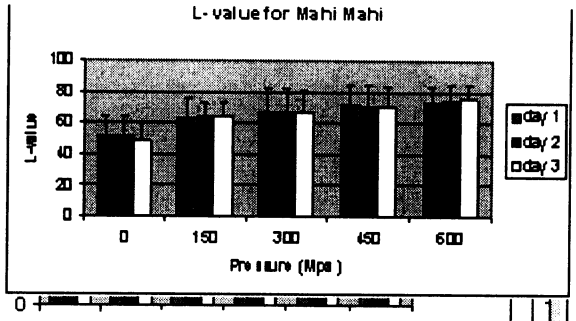
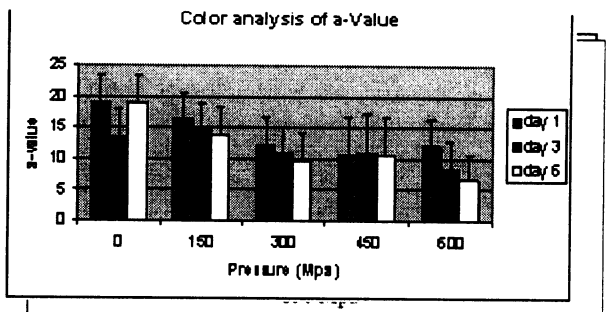
e 7. Color as represented by b-value for mahi-ma
high pressure processing and storage.



Lipid Oxidation for Mahi Mahi and Tilapia: Lipid oxidation, as represented by TBAR values, for mahi-mahi and tilapia is presented in Figure 3. Mahi-mahi processing at high hydrostatic pressures had no influence on lipid oxidation. For tilapia, there was a slight increase in lipid oxidation as pressure increased to 550 MPa.





: 6. Color as represented by L-value (left) and a-value (right) for mahi-mahi after high pressure processing orage.

Pressure (Mpa)

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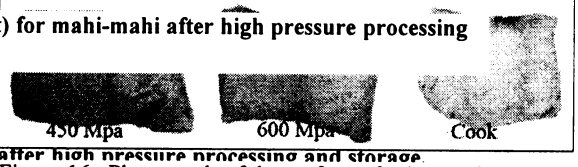
15. Color as represented by b-value for salmon after ressure processing and storage.

5. Total processing and

. TBAR levels (lipid oxidation) for mahi-mal processing and storage.

Figure 2. High pressure processing runs showing changes in t
re 10. Pictures of rainbow trout sections after high pressure tre
of storage.



after high pressure processing and storage.

Figure 16. Photograph of the surface of salmon after high pressure processing and cooking.

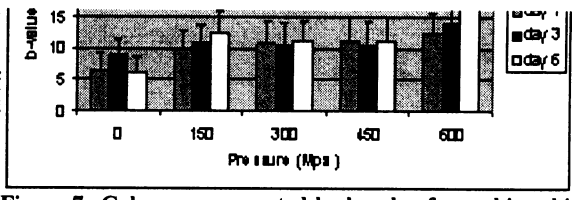
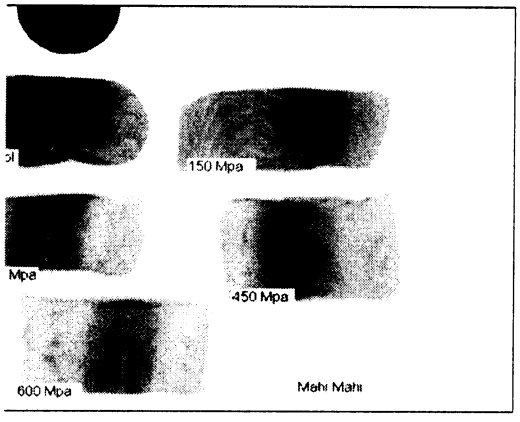


Figure 7. Color as represented by b-value for mahi-mahi after high pressure processing and storage.



8. Photograph of the surface of mahi mahi after essure processing.

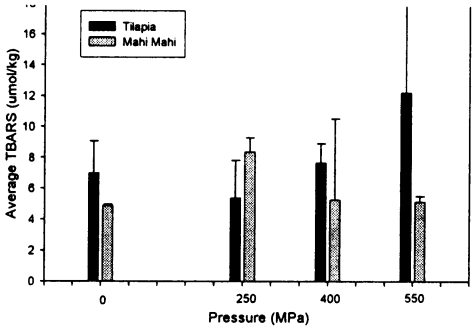


Figure 3. Lipid oxidation of mahi mahi and tilapia at various processing pressures. Values are averages±standard deviation bars, n=3.

Figure 4 shows the impact of high pressure processing on lipid oxidation through 6 days storage for mahi mahi. Lipid oxidation was examined in dark muscle tissue and the figure demonstrates that high hydrostatic pressure increased lipid oxidation. The control and 150 MPa treatments showed similar trends in oxidation with the highest oxidation occurring during day 3 storage and then decreasing by day 6 storage. The 150 MPa treatments still showed higher oxidation than the control treatment. The higher pressures (300, 450, and 600 MPa) showed similar trends with a continued increase in lipid oxidation to day 6 storage. Highest lipid oxidation occurred in the fish treated at 300 MPa.

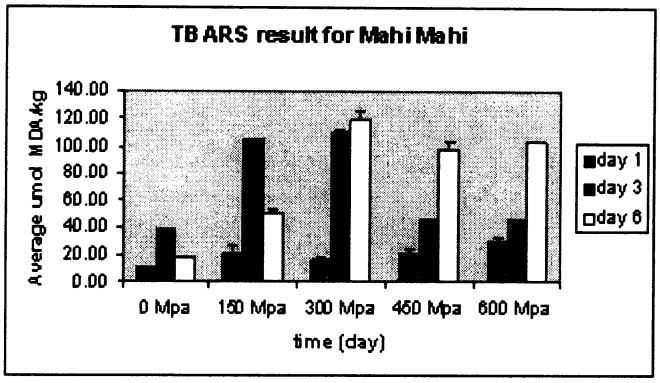


Figure 4. TBAR levels (lipid oxidation) for mahi-mahi after high pressure processing and storage.

Microbial Counts for Mahi Mahi: Figure 5 shows total aerobic plate count for mahi mahi for 6 days storage after high pressure processing. The figure represents the typical response of high pressure process on microorganisms. The higher pressures show that bacterial loads are reduced and that this type of treatment can be used to preserve seafood. The control shows the typical rise in bacterial numbers through storage. The 150 MPa process was not very different from the control while the higher pressures showed a reduction in bacterial load with pressure. The 450 showed no bacterial growth at days 0 and 3 with a slight increase in number at 6 days storage. The 600 MPa showed a slight bacteria count (>5 cfu/mL) probably due to plating contamination at 0 time and no counts at 3 days storage, but it also increased to 10 cfu/mL by day 6. The bacterial counts on day 6 probably are from cross contamination of the sample during transfer to the package for storage. Overall, high pressure processing can eliminate microbial loads and extend shelf life. However, lipid oxidation was not the result of bacterial loads as increased oxidation occurred at the higher pressures where bacterial numbers were extremely low.

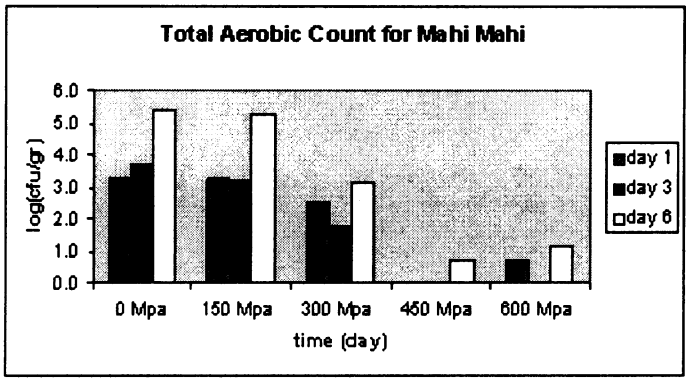


Figure 5. Total aerobic plate count for mahi-mahi after high pressure processing and storage.

Color Analysis of Mahi Mahi: Color on the surface of the fish as expressed by L-value, a-value and b-value was performed. L-value for mahi mahi increased slightly as pressure increased; however, there was very little difference between L-values during storage (Figure 6, left). The control showed the lowest L-value of all treatments followed by the 150, 300, 450 and 600 MPa treatments.

The a-value, which represents redness when positive, decreased as the pressure treatment increased (Figure 6, right). The a-value also tended to decrease as storage time increased demonstrating a loss in redness of the muscle with pressure and storage.

The b-value, which represents yellowness when positive, increased over the control for all pressures (Figure 7). This may be due to the appearance of the muscle after high pressure treatment. The muscle develops a cooked appearance as the pressure treatment increased (Figure 8). This would also cause an increase in b-value as the muscle coagulates forming a whitish-yellowish appearance. This is also reflected in the increase in L-values for the high pressure processed samples (Figure 6, left).

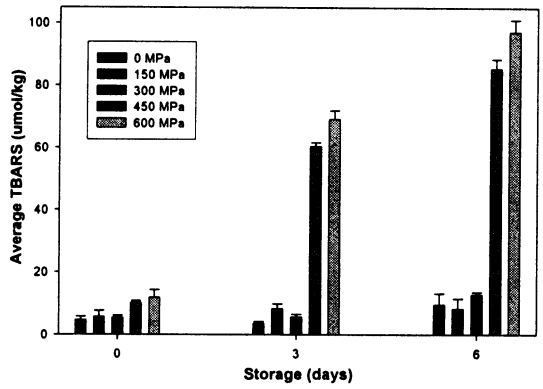


Figure 9. Lipid oxidation of rainbow trout at various processing pressures and stored for 3 days. Values are averages ± standard deviation bars, n=3.

Lipid Oxidation for Rainbow Trout: For rainbow trout, lipid oxidation increased as pressure increased beyond the 300 MPa treatment, day 0 (Figure 9). Lipid oxidation further increased at the two higher pressure treatments (450 and 600 MPa) as rainbow trout was stored at refrigerated conditions for 6 days. Lipid oxidation increased from 6 μ mol MDA/kg fish to almost 70 μmol MDA/kg fish after 3 days storage and from 13 μmol MDA/kg fish to almost 100 μmol MDA/kg fish after 6 days storage. Polyunsaturated fats are susceptible to lipid oxidation; however, further analysis of the fatty acids after treatment will be required to determine the effect on omega-3 fatty acids in rainbow trout.

Microbial Counts for Rainbow Trout: Microbiological evaluation of the treated samples is presented in Table 1. The table demonstrates that higher pressures reduce the microbiological counts by 4-6 log cycles. Processing rainbow trout at 450 to 600 MPa completely reduced bacteria. Over storage for 6 days, only the 600 MPa treatment remained free from bacteria numbers. Thus, lipid oxidation was not the result of microbial initiation but the effect of high pressure on lipid components, catalysts, etc. Extended storage studies are needed to determine the length of shelf-life after high pressure treatment.

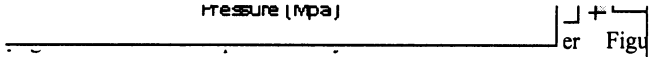
Table 1 Total plate counts for rainbow trout treated at pressures from 0 to 600 MPa. Petrifilm^{3M} plates were used for determining total counts.

Storage (days)	Pressure (MPa)				
	0	150	300	450	600
0	1.6E+04	1.3E+04	0.0E+00	0.0E+00	0.0E+00
3	2.1E+05	1.1E+05	0.0E+00	0.0E+00	0.0E+00
6	1.3E+06	3.0E+05	5.0E+03	1.5E+01	0.0E+00

Color Analysis for Rainbow Trout: High pressure treatment causes the muscle to have a cooked appearance (Figure 10), although the temperature during processing was never higher than 35^{o}C (Figure 2). Table 2 shows the L^*-, a^*-, b^*-values for the fish sections in Figure 10. L^*-values increased as pressure increased and did not change through storage; although L^*-values for day 6 storage were higher than the other two days. With an increase in L^*-values

there was a decrease in a*-value, which is related to redness. There was influence of pressure on b*-value, although b*-value was significantly higher and positive for the 6 day storage. The appearance of a cook fillet is very common in high pressure processing of muscle systems and demonstrates that high pressure can denature various proteins in muscle. Since, some of these proteins are also catalysts (prooxidants) for lipid oxidation, it is necessary to evaluate the specific prooxidant components and how they influence lipid oxidation with high pressure processing (objective IV).

igure 6. Color as represented by L-value (left) and a-value (right) for mahi-mahi after high pressure processing id storage.



igure 15. Color as represented by b-value for salmon after igh pressure processing and storage.

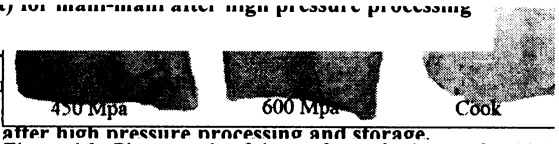


Figure 16. Photograph of the surface of salmon after high pressure processing and cooking.

ıre 4. TBAR levels (lipid oxidation) for mahi-mahi after high pressure processing and storage.

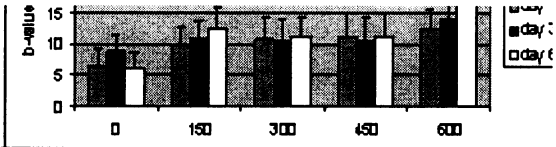


Figure 10. Pictures of rainbow trout sections after high pressure tre days of storage.

DAY 0					
Pressure	**0 MPa**	**150 MPa**	**300 MPa**	**450 MPa**	**600 MPa**
Lab L*	60.65	72.57	81.7	82.85	81.96
StdDev L*	4.53	4.85	2.91	2.63	2.98
Lab a*	11.5	10.61	4.69	2.41	2.9
StdDev a*	3.6	3.33	3.01	1.79	1.9
Lab b*	-1.23	-0.34	-2.52	-1.35	-0.68
StdDev b*	2.19	2.11	2.61	2.07	1.62
DAY 3					
Pressure	**0 MPa**	**150 MPa**	**300 MPa**	**450 MPa**	**600 MPa**
Lab L*	60.66	72.9	81.96	82.25	82.09
StdDev L*	6.62	6.27	6.33	4.03	6.92
Lab a*	14.86	13.95	3.27	2.24	1.99
StdDev a*	5.29	5.3	3.1	2.47	2.24
Lab b*	-2.82	-3.2	-4.75	-1.59	0.76
StdDev b*	4.65	3.91	4.29	2.93	2.72
DAY 6					
Pressure	**0 MPa**	**150 MPa**	**300 MPa**	**450 MPa**	**600 MPa**
Lab L*	67.77	77.69	85.85	86.81	86.6
StdDev L*	6.13	3.98	1.31	1.04	0.99
Lab a*	14.23	11.66	0.91	0.61	-0.63
StdDev a*	3.63	5.58	1.48	1.7	1.55
Lab b*	16.41	17.48	12.61	10.22	12.74
StdDev b*	2.11	2.32	4.05	3.73	3.59

Table 2 L*-, a*-, b*-values for rainbow trout sections in figure 10.

Lipid Oxidation for Salmon: Lipid oxidation, as represented by TBAR values, for salmon is presented in Figure 11. Lipid oxidation changed very little between the control and cooked treatment, and pressures (150, 300, 450 and 600 MPa) for 2 day storage samples. Lipid oxidation increased markedly after storage for 4 and 6 days. Lipid oxidation for the control increased 13-fold at day 4 storage and 20-fold at 6 day storage. Cooked samples did not increase as much as the control with a 2.5-fold and 12-fold increase for 4 and 6 days storage, respectively. The 150 and 300 Mpapressuretreatments showed very similar trends and magnitude of oxidation, and thesetreatments were the best at controlling lipid oxidation. Lipid oxidation for these treatments increased 2- and 3-fold respectively for days 4 and 6 storage. Higher pressures (450 and 600 MPa) showed similar trends as the control and cooked samples, although the magnitude of oxidation was lower for control but similar to the cooked samples.

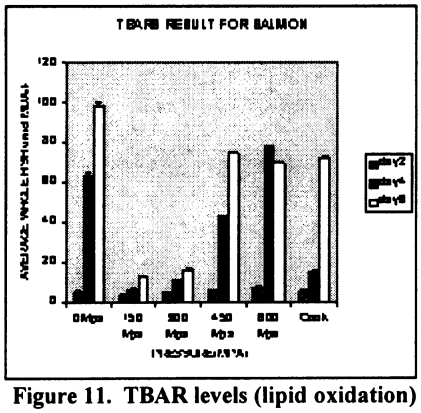


Figure 11. TBAR levels (lipid oxidation) for salmon after high pressure processing and storage.

Microbial Counts for Salmon: Microbiological evaluation of the treated salmon is presented in Figure 12. The trends were similar to those for mahi mahi (Figure 5). The control samples showed 3.5-4 log levels of bacteria and these levels were similar for 2, 4 and 6 days storage. The 150 MPa treatment showed a reduction in bacteria at 2 logs compared to 4 logs for control at day 2 storage. Further storage of the 150 MPa treatment caused an increase in bacterial level to 3 logs on day 4 and 3.5 logs on day 6 storage. The remaining pressure treatments (300, 450 and

600 MPa) showed a complete reduction in bacterial levels throughout storage. The cooked samples showed no bacterial counts throughout storage as well. This again demonstrates the effectiveness of high pressure processing on preserving seafood. Although, the quality of seafood can be improved by high pressure processing from a microbial aspect, pressures still influenced the oxidation of lipids in seafoods, which could lead to chemical spoilage problems, especially in high fat or dark muscle tissues.

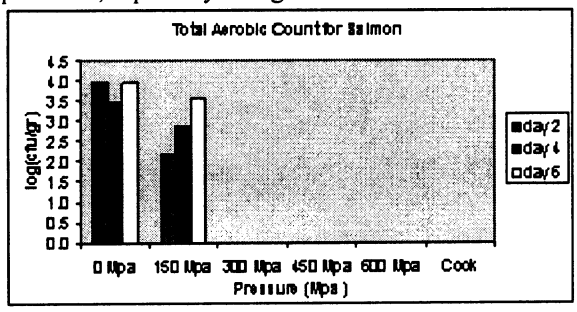


Figure 12. Total aerobic plate count for salmon after high pressure processing and storage

Color Analysis for Salmon: Color as L-value for salmon showed similar trends as that of mahi mahi (Figure 13). L-value was not different between samples at the various storage days (2, 4and 6 days). L-value increased above the control for all pressures and cooked treatments. L-values for the 150 MPa were only slightly higher than control and increased to 300 MPa and then remained constant for the remaining pressures (450 and 600 MPa). Again, the increase in L-value to a more whitish appearance probably indicates a cooked appearance on the surface of the salmon.

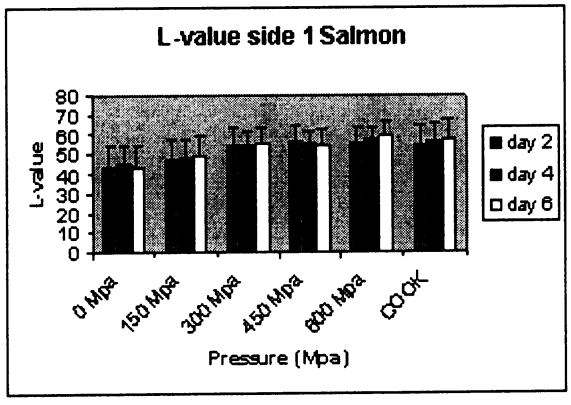


Figure 13. Color as represented by L-value for salmon after high pressure processing and storage.

The a-values decreased for pressure treatment and storage time (Figure 14). The controls had the highest a-values, which represent more redness in the muscle surface. The 150 through 450 MPa treatments were very similar in a-value magnitude and trend. The 600 MPa showed the lowest magnitude compared to the other pressures, while the cook treatment had the lowest a-value compared to all samples.

The b-values for salmon treated at different pressures are presented in Figure 15. The b-values increased as pressure increased indicating a more yellowish appearance on the surface of the muscle. The control and 150 MPa treatment were very similar while the higher pressures showed an increase in b-value. Storage time did not influence the trend in b-value as they were similar through all days. Cooked treatments were also similar to the b-values at the higher pressures. Again, the increase in L- and b-values followed by a decrease in a-values probably reflects the cooked appearance of the muscle after high pressure treatment (Figure 16).

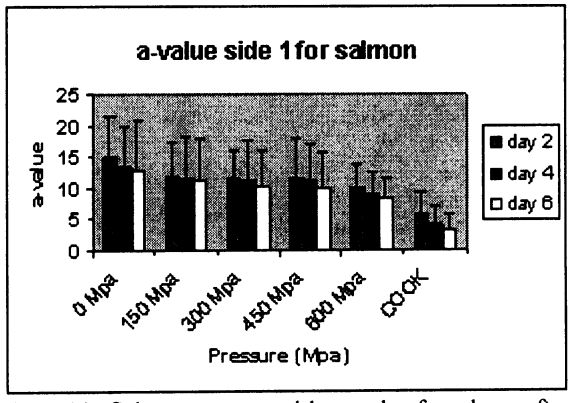


Figure 14. Color as represented by a-value for salmon after high pressure processing and storage.

Figure 16 shows a comparison of muscle treated at various high pressures and cooking with the control (0 MPa). As pressure increased, the pressure treated muscle

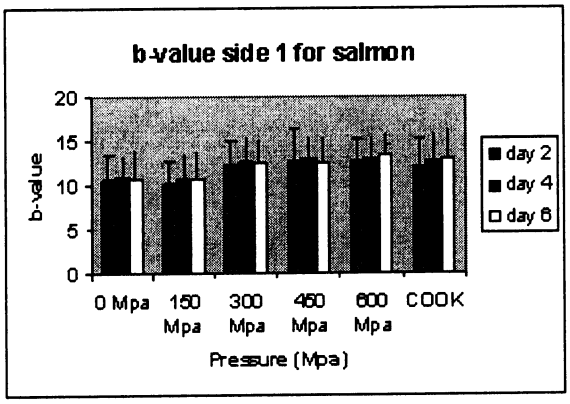


Figure 15. Color as represented by b-value for salmon after high pressure processing and storage.

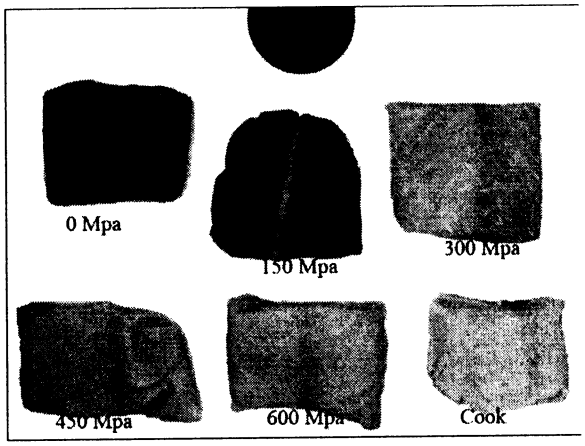


Figure 16. Photograph of the surface of salmon after high pressure processing and cooking.

became similar to the cooked sample in appearance. This again explains the changes in the L-, a- and b-values for the high pressure and cooked treated samples.

Thus, high pressure processing can reduce bacterial numbers and improve the shelf-life of seafood. Although, not a major part of this study, the shelf-life could probably be extended an additional week. Typically, the shelf-life of fresh and thawed product refrigerated is about 14 days with the first 7 days a grade A product and the remaining 7 days a grade B or C. Thus, an additional week within the grade A category would be very beneficial and economic. However, high hydrostatic pressure processing does affect the overall appearance and oxidation of lipids. This is especially important in seafood that has dark muscle areas. Further studies on how pressure influences factors that manipulate catalytic activity for lipid oxidation will be studied.

Significant Problems: The high pressure unit was down for a significant period of time throughout this work. There was a defective base vessel plate and seals that resulted in the unit not being able to maintain and hold a constant pressure over time as well as it not being able to reach maximal allowable pressures required by the project. The unit's base vessel plate and seals were replaced but still malfunctioned. The unit's manufacturer promised to sendpersonnel to fix it in early January 2004. However, four times the manufacturer of the unit made arrangements to come and repair it, and four times they cancelled the trip. Finally, in March 2004 the unit was repaired but 6 months had elapsed over the grants start date.

Additional Work: Work still needs to focus on the molecular levels of the lipids and pro- and antioxidants in the dark muscle of these fish. This work will continue with the student as this is a part of their research project.

EVALUATION

Project Goals and Objectives: Overall, this project established that various species of intact fish can undergo lipid oxidation. The major variables in this study influencing oxidation were pressure and dark muscle content. The project also demonstrated that HPP is able to reduce spoilage and probably extend the shelf-life of fresh and thawed seafood. The extent of shelf-life was not studied, however, after a week of storage at high pressures, there was no microbial counts observed. Thus, it is safe to surmise that this process could extend the product beyond the typical 14 day shelf-life, probably approaching 21 days.

The molecular basis for studying how pressure affects the pro- and antioxidants of lipid oxidation were not studied (Objectives 1, 3 and 4). The reason for this was the Stansted (England) High Pressure (HP) Unit's base vessel plate and seals were defective. This resulted in the unit not being able to maintain and hold a constant pressure over time, and also it resulted in the unit not being able to reach maximal allowable pressures required by the project. The unit's base vessel plate and seals were replaced but still malfunctioned. Stanstedstated that personnel would be sent to fix the unit in early January 2004. However, four times the manufacturer of the unit had made arrangements to come from England to repair it and four times they cancelled the trip. Finally, in March 2004 the unit was repaired and work begun to complete the objectives. During this time, personnel involved with the project were able to master the isolation techniques necessary to carry out objectives 1, 3 and 4. Because of this delay, twice we did request a no cost extension but it was never formalized. Therefore, a decision was made to stress objective 2 and evaluate high pressure processing of fillets from various seafoods (mahi mahi, rainbow trout, tilapia and salmon). The goal here was that if intact muscle fillets did not demonstrate lipid oxidation, then it would be pointless to move into the other objectives.

Project Dissemination: The results from the project will be presented at national seafood meetings and through the submission of a manuscript to the Journal of Food Science. The work will be presented at the 2005 National Meeting of the Institute of Food Technologists (IFT)in New Orleans, LA as either a talk or poster in the Aquatic Foods Division. Additionally, our extensions specialist Dr. Steve Otwell will receive a copy of this report so that he can distribute the information to those stakeholders he fills need this information.

REFERENCES