THESIS

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Ph.D. DISSERTATION

INCREASING THE SHELF LIFE OF FISHERY PRODUCTS WITH COMPLEX, GENTLE PROCESSING TECHNOLOGY

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1. BACKGROUND OF THE WORK, OBJECTIVES

The processing technology of the domestic fisheries sector lags significantly behind the development of other areas of agricultur. Both domestically and internationally, the marketing of the main products of the sector, carp,in accordance with the market needs is unresolved.

The aim of the research is to examine the most important product of the Hungarian aquaculture sector, the entire carp processing vertical, and to reveal at what point in the process and how to improve the microbiological and organoleptic quality of an otherwise very short-lived raw material to produce a safe food product.

I aimed to achieve the goal by answering the questions of the following, successive steps.

1. What is the impact of transport stress on freshly harvested and then transported live fish on the quality of the meat? The aim of the experiment was to determine the color, pH, texture, water holding capacity of the different groups of samples, and to determine the glycogen, cortisol and cortisone contents of fish meat and liver.

2. After exploring the effect of transport stress on fish meat, I sought the answer to how the application of the appropriate slaughtering technology, as the next step in the processing, affects the quality of fish meat. In the experiment, my aim was to investigate whether the freshness of fish meat changes during the storage, when using gentle cutting technologies, such as electric shock or the Japanese ikejime method, and whether the applied technologies have different effects on key quality parameters. My further goal was to investigate how different pre-cooling processes can prolong the freshness and thus the shelf life of fish meat. To evaluate these, it is advisable to study the changes in the color and pH of the fish meat, the changes in the sarcoplasma by gel electrophoresis, and the changes in the amount of ATP degradation products.

3. The aim of my experiments is the development of a new, gentle processing technology suitable for the production of ultra-fresh products in fish processing. The main question is whether microbial contamination, a critical problem in fish processing technology, can be significantly reduced by electrolyzed active water treatment, which is still used in a few areas in the food industry. In the experiment, I compared acidic and mixed electrolysed active water from two different manufacturers. The study covered the

effect of the treatment during immediate and longer storage, for which, in addition to measuring the total germ count and Enterobacteriaceae number, I also performed a challenge test.

4. I investigated the additive effect of electrolyzed active water and other bactericidal agents and their organoleptic changes on fish meat. In addition to acidic and mixed electrolysed active waters, I used lysozyme enzyme and lactic acid and analyzed how tap water washing after the application of electrolyzed active water affects the effectiveness of the treatment. For this, in addition to measuring the total germ count, Enterobacteriaceae number, mesophilic anerobic microbial count, TBA number, and the amount of residual chlorate and perchlorate, I also performed sensory evaluation.

5. In addition to the electrolyzed active water treatment, I also investigated the applicability of the HHP treatment already used in other areas of the food industry, to improve the shelf life of both raw meat and the finished product. During these experiments, I examined the effect of HHP treatment on the pH, driploss, protein fractions, microbiological and organoleptic properties of carp meat.

The research is part of a supported research and development project. My task was to compile the tender application material of the research topics, to formulate the research goals. I participated in the elaboration of the tender topics, as a research leader I led the research group, and I participated in the specific analytical studies of the sub-topics, the evaluation of the results and the formulation of the conclusions.

1. RESULTS

In my research, I was looking for the answer to whether the freshness, organoleptic and microbiological quality of fish products can be significantly improved by using relatively recent technologies, which are less common in the industry. The research ranged from the transport and slaughter of live fish to the handling of the finished fish product.

In the first experiment the effect of live fish harvest, transport and resting on fish meat quality indicators were investigated. The transport experiments were performed between Akasztói Halgazdaság (Akasztó) and The FIshmarket Ltd. (Budaörs). Samples were taken after each processing step. The fish meat was examined for cange of color in the CIELab color space, final pH and glycogen content, hardness (F, N) and water holding capacity (WHC, mm / g).

In order to examine the stress caused by transport, the glycogen, cortisol and cortisone levels of fish meat and fish liver were also measured. As a result of resting before slaughter, the flesh of the fish became more light and red and vivid in appearance than the individuals slaughtered without resting. Based on the results, the resting of fish for the reduction of stress levels has more impact on the meat color, than the time of delivery. The final pH values measured in the fish meat showed a negative correlation with the amount of glycogen measured in the fish meat. There was a significant difference in the consistency of fish meat in relation to the transport times.

The meat of fish exposed to the stress caused by the long-time transport proved to be twice as hard as the meat of individuals transported for a short time. The greatest amount of glycogen was measured in fish slaughtered locally in the processing plant (2.6 mg / kg). Cortisol, also called the stress hormone, was found in higher levels in case of long distance transport (124.7 μg / kg), which can be associated with stress suffered during transport. The glycogen concentration of 0,1-0,3 was measured in the fish liver, showing that more significant levels of glycogen is stored in the fish meat than in the liver and in case of stress, fish first mobilizes its glycogen stored in its meat.

According to the results of the color test, the values of L* brightness color factor were higher in case of the ike-jime stunning, i.e. the color of the fish meat was found brighter. This can be particularly beneficial, as fish fillets are also generally characterized by a light color that gives the consumer the appearance of a fresh product. The values of the color factor a*

were similarly higher in case of the ike-jime stunning, i.e. the color of the fish meat in the color space fell more in the red range. No such changes could be observed for the color factor b*.

When examining the pH, the values were found higher in case of the ike-jime method, especially in the first 30-60 minutes, which may be favorable for freshness. However, during storage, the values equalized or changed in the same way, no significant differences could be noticed. In the case of color and pH values, it should be mentioned that it can be very large deviation between the individual fish samples, so that only clearly definite conclusions can be drawn by repeating the tests several times and validating the results.

The sarcoplasm soluble proteins form large proportion of cell proteins. The majority of sarcoplasmic proteins are enzymes, catalyzing power generating reactions. The most relevant proteins of the sarcoplasm in respect of food industry are myoglobin and hemoglobin as they give the color of the meat. No significant difference was observed between the two samples in the sarcoplasmic proteins, examined by gel electrophoresis.

The application of ike-jime slaughtering method was found beneficial in case of carp, as the autolytic degradation of meat constituent proteins proceeds slower, therefore the meat freshness indicating K index was found lower than in case of the electrically stunned samples. Furthermore the IMP content of the meat was found higher during the whole storage. Based on the above, I recommend the use of ike-jime stunning during slaughter of fish in order to produce better quality fish meat.

The aim of the experiment performed with electrolyzed active water was to establish a new novel processing technology, resulting in ultra-fresh fish products. With the studies carried out, I was looking for the answer to whether microbial contamination, which is a critical problem in fish processing technology, can be significantly reduced by electrolyzed active water treatment, which is still used in a few areas in the food industry. It was a key issue to demonstrate that the treatment has an inhibitory effect on the growth of microbes typically found in fishery products.

The microbiological results of the study demonstrated that electrolyzed active water is effective in reducing microbial counts on fish surface and can be used effectively against human pathogens such as Salmonella typhimurium and Listeria monocytogenes. The results show that from chlorine concentration and exposure time, concentration affects the outcome of the treatment more significantly. Analyzing the data from the dilution series and the

experimental matrix of the treatment time with a post-hoc test, active water with a chlorine concentration of 120 ppm proved to be the most effective.

The practical usability of the technology was tested with a storage test. The aim of the study was to determine whether the immediate germ-reducing effect of the treatment also had an inhibitory effect during the storage of ultra-fresh fish meat. The effectiveness of agents from an active water generator from two different manufacturers were tested. It can be stated that the electrolyzed oxidizing aqueous treatment has an inhibitory effect on the growth of microbes present on the surface of fish meat. During the storage test, the difference in IgN was 0.6–1.04 compared to the control in favor of the active water-treated product. The effectiveness of the agent could only be significantly demonstrated in the case of water from the equipment of one of the manufacturers. This may indicate that the electrolyzed active water generated in advence loses its effectiveness over time. The mixed active water used for the test was used 48 hours after its production in Germany and the transport conditions are also uncertain. In order to use electrolyzed active water efficiently, it is advisable to produce it locally.

Although electrolyzed active water has been shown to significantly increase the shelf life of carp meat, treatment has caused only a one-order decrease in the total germ count. The aim of the next experiment is to try to increase the shelf life of carp fillets by combined treatments using electrolyzed active water and lysozyme enzyme as well as lactic acid. Fresh skin-on fillets from carp were treated with a 60:40% mixture of anionic and cationic electrolyzed active water at a concentration of 100 ppm chloride ion and a 0,5% concentration of lysozyme enzyme and a 2% solution of lactic acid, as well as anionic electrolyzed active water was combined with 0,5% lysozyme enzyme and with 2% lactic acid solution. During the storage test, the samples were subjected to chemical (TBA, chlorate), microbiological (total microbial counts) and organoleptic tests to examine the effect of the new combined method on shelf life and meat quality when stored at 2 ° C. Based on the microbiological studies, I can clearly state that the shelf life of the skin-on fish fillet was increased from 4 days to 10 days compared to the control samples as a result of the combined surface treatment. Based on sensory tests, treatment with a 60:40% mixture of anionic and cationic water with a chloride ion concentration of 100 ppm does not cause a significant change in the quality parameters of fish meat, as opposed to acidic water treatment with a chloride ion concentration of 100 ppm. In the latter case, residual chlorate level above the permissible limit can be detected in the treated product.

In the next experiment, I sought to answer whether pure aqueous washing after acidic electrolyzed active water treatment could reduce residual chlorate and perchlorate, and whether lactic acid or lysozyme enzyme compensated for the loss of efficiency resulting from the washing step.

For the control group, the untreated slices were vacuum packed in PP foils. The second group was washed with tap water. The third group was treated with acidic (pH = 2.5) electrolyzed active water with a concentration of 100 mg / kg chlorine ion, which was prepared with a REDO Pure 250 active water generator. The fish fillets were placed in 25 liters of active water for five minutes and stirred every minute. The fourth group was washed with tap water after the electrolyzed acidic active water treatment. The fifth group was placed in acidic electrolyzed water and then its surface was sprayed with 0.5% lysozyme enzyme. The sixth group was placed in acidic electrolyzed water, washed with tap water, and then treated with lysozyme enzyme solution. The seventh group was sprayed with 2% lactic acid solution after acidic electrolyzed active water treatment. The eighth group was placed in acidic electrolyzed water, washed with tap water, and then treated with a lactic acid solution.

Both acidic electrolyzed active water and the combined treatment effectively increased the shelf life of the samples, causing a difference of 2.4-3.1 log CFU / g compared to the control by the end of the 7-day storage. The measured residual chlorate content exceeded the legal threshold, but washing the samples resulted in values below the theoretical threshold recommended by EFSA for food (EFSA, 2015). The preservation methods used did not adversely affect the organoleptic properties of the samples. The use of combined treatment effectively compensated for the reduction in efficiency caused by washing.

Like electrolyzed active water treatment, high hydrostatic pressure treatment is another, even less common treatment. In this series of experiments, we examined the effect of this treatment on fish meat. In doing so, we sought to select the appropriate pressure treatment time value that does not cause sensory and structural changes in the fish fillets, which retain their natural freshness, while at the same time increasing the quality retention time. During the performance of the experiments, we continuously examined the main quality parameters of the pressure-treated fish fillets such as pH, color, drip loss, frying loss, total germ count and protein structure transformations.

Based on the results of the first series of experiments, treatments at pressures of 400 and 600 MPa already significantly change the quality of the fish fillets, they lose their original

freshness and their visual enjoyment value is greatly reduced. They also suffer significant drip loss during treatments and baking. However, lower pressure treatments can be used to achieve the desired result. Examination of the immediate effect of the combined treatment with electrolyzed active water on the number of microbes showed that the microbicide effect of the treatment significantly increased with increasing pressure, and the combination of the treatment with electrolyzed active water further increases the effectiveness of the treatment.

In the second series of experiments, where I examined the previously established possible pressure range in more detail, we found that up to 250 MPa the external appearance and protein structure of fish fillets do not change significantly, but from a microbiological point of view a higher pressure value would be more sufficient.

In the third series of experiments, I therefore included a series of even shorter pressure treatment times (2 min) to examine whether the desired microbiological stability could be achieved while maintaining freshness. The test results show that the pressure values used during the pressure treatments have a much more influential effect on the changes in the fish fillets than the duration of the pressure treatments. The external appearance of fish fillets changed only minimally up to 250 MPa. It can be stated that by applying these parameters, no significant changes have yet taken place in each of the quality parameters, however, it significantly increased the microbiological stability of the samples.

In the fourth series of experiments, based on the first three trials, I chose the treatment of 250 MPa for 5 minutes to perform the storage test. Storage was done without packaging for 5 days, in case of vacuum-packed fish fillets for 10 days. In case of unpackaged fish, there were no significant changes in quality parameters, but drip loss was high even here. During the 10-day vacuum-packed storage, the amount of drip loss was reduced, and the quality parameters also suggested the fresh nature of the fish fillets.

The results of the microbiological test support the literature, accordingly, the HHP method reduced the total number of aerobic germs by 2 orders of magnitude compared to the initial state. It can be observed that in the case of storage without packaging for 5 days, the number of germs increases slightly as a result of the treatment, but does not exceed even an order of magnitude. However, the number of microbes in the case of vacuum-packed fish fillets did not increase in 10 days. Thus, it can be concluded that as a result of the process, the shelf life can be extended for both unpackaged and vacuum-packed fish.

In a previous series of experiments, it was found that HHP treatment is not suitable for treating ultra-fresh fishery products without altering organoleptic properties. The aim of this series of experiments is to investigate the effect of HHP treatment on prolonging the shelf life of a finished product. I performed the experiment on smoked carp fillets.

Examining the effect of high hydrostatic pressure treatment, it can be said that no significant changes in the quality factors of smoked carp fillets occurred directly as a result of the treatments. For pH values, the 600 MPa pressure treatment only slightly increased the pH of fish fillets. In addition, during storage, the pH of the control samples decreased significantly after day 7, while the pressure-treated samples (450 MPa, 600 MPa) fluctuated at the initial value. Statistical analysis also confirms that pressure treatment significantly helped to maintain the initial pH of the samples compared to the control samples.

Examining the coloration, it can be said that no changes occurred as a result of the pressure treatment for any of the color factors (L *, a *, b *). Examining the effect of pressure treatment immediately after the treatments, the sensory results showed that no major changes were found in the 450 MPa pressure-treated samples, only the odor intensity of the 450 MPa pressure-treated samples decreased slightly and the texture of the fish was assessed as softer. No differences were found in case of the samples treated with 600 MPa.

During the 21-day storage, it can be concluded that no major differences were detected in the essential properties of the pressure-treated refrigerated samples and the control samples. The two traits, which show a slight difference, were unfortunately no longer adequately evaluated after day 7, as for safety reasons, tasting of the control samples was not obligatory. However, during the 26-day evaluations, the pressure-treated samples were no longer tasted either, as the reviewers considered that they showed deteriorating properties. Overall, however, by day 21, the pressure-treated samples met sensory expectations.

Microbiological results showed that the 450 MPa pressure treatment was able to reduce the initial total germ count of the samples by one order of magnitude and the 600 MPa pressure treatment by two orders of magnitude. During storage, the control samples stored at 10 ° C approached the total germ count of $N = 10^7$ TKE / g already on the 7th day, these samples had a sour smell and deteriorated during the sensory examination. During storage, the fastest increase in total germ count was shown by the control samples, followed by the results of the

450 MPa and then the 600 MPa pressure samples. Thus, pressure treatment not only reduced the initial germ count but also increased the shelf life. The effect of the two different storage temperatures is also reflected in the results.

The results of storage at 5° C were consistently lower than the results of samples stored at 10° C. Samples treated with a pressure of 600 MPa were about one order of magnitude after day 14, while samples treated with a pressure of 450 MPa were about two orders of magnitude. Thus, in order to increase the shelf life during the production of safe smoked carp fillets and to take into acount the fluctuations in storage temperature under real conditions, it is recommended to use a high hydrostatic treatment at 600 MPa to safely preserve the shelf life specified by the smoked carp fillet manufacturer. Overall, it can be concluded that the high hydrostatic pressure treatment did not affect the physical and organoleptic characteristics of the smoked carp fillet. However, it caused a significant beneficial effect in the microbiological state.

2.2 NEW SCIENTIFIC RESULTS

1. In the study of carp harvesting and live transport, based on the changes in the cortisol hormone content, texture, color and water holding capacity of fish meat, I found that the increased stress caused by harvesting and live transport triples the cortisol hormone level and negatively affects the texture, color and water content of fish meat. There is a strong relationship between cortisol hormone concentration and meat quality parameters such as texture, color and water holding capacity. I found that after the stress effect, 48 hours of resting before slaughter is required with a constant water temperature and 10% water change per day to achieve the lowest cortisol hormone levels and the best meat quality.

2. In the case of K index, calculated from the ratio of IMP, inosine and hypoxanthine and these compounds during the enzymatic degradation of ATP in carp meat, I found that when using rapid mechanical destruction of the central nervous system (ikejime stunning technology) the K index value remains 10% lower during storage, than with the use of electrical stunning. Thus, using rapid mechanical stunning, the concentration of IMP is significantly higher and the concentration of hypoxanthine, which causes unpleasant off-flavors, is significantly lower. After slaughter, the pre-rigor phase in carp meat elongates, rigor-mortis occurs more slowly, and carp meat can be stored for a longer time in terms of autolytic decomposition.

3. In the case of comparing cooling technologies after carp slaughtering, I found that cooling in icy water chilled the 20 ° C carp carcass in 90 minutes, while air cooling reduces it below 2 °C in 16 hours. Based on the effect of the cooling method on the concentration of IMP, inosine and hypoxanthine in the fish meat and the change in the K index value calculated from the ratio of these compounds, I found that faster ice water cooling of the carp significantly slows down the phosphatase enzyme in the initial phase. This can prolong the shelf life of carp meat by 3-4 days in terms of autolytic decomposition, and significantly affects the taste, color and texture characteristics of fish meat.

4. Based on the microbiological measurement of the samples in the matrix of treatments with active water in the time interval between 30 seconds and 10 minutes, with chloride ion concentration between 60-180 ppm, I found that chloride ion concentration significantly reduces the microbiological load of the surface of fish meat by more than an order of magnitude. In the studied range, the treatment time has no significant effect on the germ count of the tested microorganisms.

5. During the treatment of carp with anionic electrolyzed active water (pH 2.5) with 100 ppm chloride ion concentration for 5 minutes, electrolyzed active water and 2% lactic acid and treatment with combinations of lysozyme enzyme solutions with 0.5% concentration, I found that without a significant change in physical and organoleptic properties, a reduction of 2.5–3 orders of magnitude in the number of microbes on the carp fillet surface could be achieved compared to the control samples. Regarding chemical effect, application of electrolyzed active water treatment, combined with with lysozyme and lactic acid and the addition of a rinsing step with tap water, resulted in 0,29 mg/ kg chlorate and 0,013 mg / kg perchlorate levels while maintaining the significant germ-reducing effect of 1.5-2 orders of magnitude of the treatments. This result is below the theoretical limit (MRL) of 0.7 mg / kg recommended by EFSA for food.

6. Based on microbiological, chemical, physical and organoleptic studies of carp fillet and hot smoked carp fillet in the combination of high hydrostatic pressure treatment (HHP) and anionic (2.5 pH) electrolysed active water with 100 ppm chloride ion concentration, I found that treatment of HHP above 250 MPa has an additive effect on microbial death, but adversely affects the sensory results of raw carp fillets. In the case of hot-smoked (60 °C, 90 min) carp fillets, protein denaturation already takes place during the smoking process, so HHP treatment at 600 MPa results in a microbiologically stable fish fillet for more than 21 days without significant change in its organoleptic and physical properties.

2. *RELEVANT PUBLICATIONS*

IF-es folyóiratcikk:

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https://doi.org/10.1155/2020/4861471

Salamon, B., Tóth, A. **Palotás, P.**, Südi, G., Csehi, B., Németh, Cs., Friedrich, L., 2016. Effect of high hydrostatic pressure (HHP) processing on organoleptic properties and shelf life of fish salad with mayonnaise. Acta Alimentaria 45, 558-564. IF 0,384
https://doi.org/10.1556/066.2016.45.4.13

Lehel József (Lehel József Gyógyszertan, toxikológia, élelmiszer-higiénia) ÁTE/ÉBJI/Élelmiszer-higiéniai tanszék ; Yaucat-Guendi Rebecca ; Darnay Lívia (Darnay Lívia Élelmiszertudomány) ÁTE/ÉBJI/Élelmiszer-higiéniai tanszék **; Palotás Péter (Palotás Péter Élelmiszer) SZIE/ÉTK/Hűtő és Állatitermék Technológiai Tanszék ;** Laczay Péter (Laczay Péter Élelmiszer-higiénia, állatorvosi gyógyszertan,) ÁTE/ÉBJI/Élelmiszer-higiéniai tanszék
Possible food safety hazards of ready-to-eat raw fish containing product (sushi, sashimi)
CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION (1040-8398 1549-7852): (2020)
Folyóirat szakterülete: Scopus - Food Science Helyzete: D1
Folyóirat szakterülete: Scopus - Industrial and Manufacturing Engineering Helyzete: D1

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Palotás, P., Salamon, B., Südi, G., Csehi, B., Tóth, A., Staszny, Á., Friedrich, L., 2015. Photometric measurement of ATP decay products to determine the effects of different cutting technologies ont he quality of carp. Food Science Conference 2015, Corvinus Uni. Bp., pp 191-194, ISBN 97896350366035

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Jónás, G., Csehi, B., **Palotás, P.** Darnay, L., Pásztor-Huszár, K., Friedrich, L., 2015. High hydrosttic pressure treatment and concentration effects on functional properties of meat batter. Food Science Conference 2015, Corvinus Uni. Bp., pp 112-116, ISBN: 9789635036035