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FAT OXIDATION, PROTEIN DEGRADATION AND COLOUR OF RAINBOW TROUT (ONCORHYNCHUS MYKISS) MEAT DURING 3 MONTHS OF FREEZER STORAGE

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ABSTRACT

The aim of the work was to analyze the effect of storage in freezing conditions (-18 ° C) during 3 months of storage on the formation of malondialdehyde MDA, TVB-N (total volatile basic nitrogen), and colour. Rainbow trout up to 500 g (RT) aged 1-1.5 years (n=15) and trout (RT1) up to 3.5 kg aged 2-2.5 years (n=15) were included in the experiment. The stated weight of the fish is already after processing. After slaughtering the fish, samples were taken from each fish for analysis of meat colour in the CIE colour space with a KONICA MINOLTA 2600D (L*, a*, b*) on the 1st day after slaughter and after the 1st, 2nd, 3rd month of storage. The content of TVB-N and MDA was measured parallel. For both weight categories of trout, after three months of freezing, we observed significant (RT1- P≤0.05; RT- P≤0.05) increase in TVB-N compared to the first day of measurement. Malondialdehyde content after three months of freezing was significantly (P≤0.05) higher than at the first measurement in both weight categories of rainbow trout. In the RT experimental group, trout meat after three months of freezing was significantly (P≤0.05) lighter (L*) and less yellow (b*). The meat of the RT1 group after 3 months of freezing storage was significantly (P≤0.05) darker , less red and less yellow.

Keywords: fish meat, meat degradation, malondialdehyde, TVB-N, meat colour

INTRODUCTION

Fish meat, as an animal protein source, is characterized by a protein content of 15-20% **(Pipová *et al.*, 2006).** The proteins of this animal species contain essential amino acids, which are in a balanced and favorable ratio for human nutrition. We describe them as full-value and also easily digestible **(Buchtová, 2001).** Easy and quick culinary treatment of fish meat is connected with the fact that fish meat contains minimal amounts of connective proteins **(Buchtová, 2001).** In the case of fish, emphasis is placed on histamine (biogenic amine) produced by enzymatic decarboxylation, which is associated with unwanted allergies in sensitive people **(Pipová *et al.*, 2006).** Fish meat proteins contain essential amino acids that improve the overall nutritional quality of a mixed diet. A portion of 140 g of fish can provide about 50-60% of the daily protein intake needed by an adult **(Balami *et al.*, 2019).** Fish meat proteins are highly digestible and rich in several peptides and essential amino acids not found in terrestrial animal meat proteins, such as methionine and lysine **(Tacon and Metian 2013).** Proteins from various fish such as bonito, salmon, mackerel, herring and cod have shown anti-inflammatory properties. Proteins from cod support the growth and regeneration of skeletal muscles after injuries compared to peanut protein and casein **(Khalili Tilami *et al.*, 2017).** All studies indicate that fish consumption has a positive effect on human health due to its lipid content and protein/peptide composition. In addition, some amines such as spermine and spermidine are very important for cancer prevention **(Wang *et al.*, 2017).** The oxidation of fats in food is closely related to the content of unsaturated fatty acids **(Jurčaga *et al.*, 2021).** Fish, both freshwater and marine, are an extremely rich source of basic human food items. Marine fish species are mostly enriched with high levels of n-3 PUFAs, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), making them an excellent source of these nutrients in the human diet **(Zang *et al.*, 2020).** The fatty acid composition of fish varies depending on factors such as species, diet, and environmental factors such as salinity, temperature, season, geographic location, and whether the fish is wild or farmed **(Balami *et al.*, 2019).** Fish are a major source of the n-3 PUFAs EPA (C20: 5 n-3) and DHA (C22: 6 n-3), which are attributed many health effects. Meat from other animal species is poorer in these PUFAs but is the main source of PUFAs in people who do not eat fish **(De Smet, 2012). Osman *et al.* (2001)** examined the fat content of various types of marine fish, stating that most fish had a fat content below 5%. **Komprda *et al.* (2003)** states that the cholesterol content in meat of carp is 73.5 mg. $100g^{-1}$. Cholesterol content in fish meat is variable. Marine fish species have a cholesterol content of 37.1-49.1 $mg.100g^{-1}$ **(Osman *et al.*, 2001).** Fish lipids have shown a positive effect in the prevention of certain diseases such as cardiovascular diseases. Omega-3 fatty acids found in oily fish are known to be essential for the growth of children and prevent coronary heart disease **(Balami *et al.*, 2019).** Excessive n-6 PUFA intake is therefore associated with adverse effects on human health, such as cardiovascular disease, diabetes, hypertension, depression, neurological dysfunction, and immune disorders **(Williams, 2000).** Although fish meat contains a large amount of unsaturated fatty acids and is generally considered healthy, it is necessary to point out the storage period in stores, whether the fish is chilled or frozen. Fish stored for a long time may be sensorially unacceptable.

The quality of proteins and their denaturation in muscle tissue is closely related to postmortem changes in animal meat, which are the most important part of quality meat production **(Čuboň *et al.*, 2006).** Changes in meat take place in the following stages: For *rigor mortis* - less cold losses, less drain losses, in freezing temperatures. *Rigor mortis* – lasts until disruption (fragmentation) of actin and myosin fibers. Self-ripening -y (proteases) decrease the weakening of the structures of myofibrillar fibers. Autolysis – molecular breakdown of cell membranes and their subsequent degradation **(Čuboň *et al.*, 2020).** Proteolysis, the oxidation and phosphorylation of proteins, play important roles during meat maturation and contribute to quality variability **(Carlin, 2018).** During storage and transport, fish meat deteriorates through microbial and enzymatic reactions. Chemical and biological changes caused by the action of enzymes, especially proteases. Protease-mediated protein degradation should be minimized in order to preserve fish meat quality. In addition to commonly used preservation methods such as freezing or refrigeration, additives that reduce protease activity can be used. Food grade protease inhibitors (PI) can be used to obtain premium quality fish meat and fish products **(Singh and Benjakul, 2018).** Amino acid side chains in myofibrillar proteins undergo modifications due to oxidative stress, leading to the formation of new protein-protein cross-links in their structures, but the overall level of fixed charge groups attached to the peptide backbones is also altered **(Bao and Ertbjerg, 2018).** The content of total volatile basic nitrogen (TVB-N) is an essential direct indicator for evaluating the freshness and safety of meat. TVB-N is an alkaline nitrogen-containing substance that is formed by the breakdown of proteins due to enzymatic degradation and microbial action during the spoilage of animal-based

foods. TVB-N in meat contains ammonia, trimethylamine and dimethylamine. During spoilage, TVB-N can combine with decomposed organic acids, forming a basic nitrogen salt (+ NH4 · R−), which can increase the content of TVB-N in meat **(Li *et al*., 2019).** TVB-N content in meat is also key in measuring meat quality and freshness during storage **(Lee *et al*., 2018).** TVB-N, an important index representing the content of ammonia, trimethylamine and dimethylamine in measured samples, is widely used in determining the quality of meat and meat products. TVB-N analysis generally has some disadvantages, such as complex compound search, time-consuming, repeatability, and environmental pollution **(Tang and Yu, 2020).**

Fats are sensitive to oxidation, which is the main non-microbial cause of spoilage in animal products. Degradation of fats begins with obtaining the product, in the case of meat, by killing the animal and ends with consumption. The degradation process is affected by storage, processing, handling and transport, therefore care should be taken to control ideal conditions during these operations **(Dominguez *et al*., 2019).** Oxidation reactions not only reduce the nutritional value of meat due to the loss of essential substances, fatty acids and vitamins. Furthermore, these include changes in colour, texture and appearance, aroma and taste that affect the consumer's sensory perception **(Purinos *et al*., 2011).** The degradation process of lipid oxidation is triggered by the presence of oxygen, light and lipoxygenase enzymes. During the storage and processing of meat, oxidation is caused by improper temperature, the presence of light, the presence of heavy metals, and also the presence of technologically undesirable microorganisms that produce enzymes. According to the source of oxidation, it is divided into autoxidation and photo-oxidation **(Shahidi and Zhong, 2010).** The main factors that affect the oxidation of lipids in meat are the fat content and the composition of fatty acids, since fatty acids are the substrate of oxidation processes. In meat, lipids are organized into triglycerides and phospholipids, cholesterol or vitamins. The amount of intramuscular fat is directly correlated with the content of triglycerides, because these are reserve lipids (≈ 95% of meat lipids) **(Pereira and Abreu, 2018).** The products created by oxidation can be divided into primary and secondary, which are further indicators of oxidation. Primary oxidation products are, for example, hydroperoxides and conjugated dienes **(Niki *et al*., 2005).** Further oxidation is supported by the reaction of secondary oxidation products with other substances contained in the meat. The reaction of amino acids with reactive side chains results in the formation of carbonyl compounds **(Baron, 2014).** A wide range of secondary products includes compounds such as malondialdehyde (MDA), propane and hexanal, which acquire the yellowing of meat, while malondialdehyde can be considered as an indicator of its freshness **(Ross and Smith, 2006).** Malondialdehyde (MDA), a three-carbon compound that results from the breakdown of peroxidized PUFAs, is one of the major products of lipid peroxidation. As a result, a substantial amount of aldehydes will occur in lipid oxidation **(Pereira and Abreu, 2018).** The most common oxidation indicator and the most represented aldehyde, a product of secondary oxidation of lipids, is malondialdehyde. The final products (malondialdehyde / 4-hydroxynonenal - 4-HNE) of oxidation are less significant than the primary products (hydroperoxides). The ability of MDA to alter various biological macromolecules may contribute to its toxicity and its mutagenic and carcinogenic properties **(Reitznerová *et al*., 2017).** Fat oxidation primarily reduces the quality of the product and negatively affects mainly the structure, colour and sensory properties of meat and products **(Bobko *et al*., 2015)**.

The aim of study were analyzed content of MDA, TVB-N and meat colour of rainbow troth two groups up to 500g and 3500g. Analyzed parameters were analyzed at 1st day, 1st, 2nd and 3rd month of storage.

MATERIAL AND METHODS

Two types of fish were used in the experiment, namely (RT) rainbow trout up to 500g (1-1.5 years) and (RT1) rainbow trout up to 3.5 kg (2-2.5 years). We chose these fish because they are the basic commercial size of fish. The indicated weight of the fish is after processing. From each analyzed species, samples (n=15) were taken from the back muscle. The fish were killed and processed in the company's processing plant according to the applicable legislation: stunning – electrically, dissecting – up to 500g by machine (BOLETO), trout up to 3.5 kg (by hand), washing fish from dirt with water, rapid cooling in flake ice, vacuum packaging (MULTIVAC), shock freezing (-36°C), storage (-18°C).

Analyzes were performed within 24 hours of slaughter and then repeated every 30 days from the last analysis for 3 months.

Determination of meat colour

Meat colour was determinate according to **Debrecéni *et al*. (2018)** 24 hours after death and then 1st, 2nd, and 3th months of storage using a Minolta colourimeter (2600D). The Commission Internationale de l'Eclairage (1975) determined the following colour coordinates: L* (luminosity, white ± black), a* (redness, red ± green) and b* (yellowness, yellow ± blue). These values were recorded from the average of five measurements on the dorsal muscle surface from the gill arch to the caudal fin.

Determination of Oxidative Stability

The oxidative stability was measured according to **Jurčaga *et al.* (2021)**, was based on measurements of the malondialdehyde (MDA) concentration by thiobarbiturate test using a 2-thiobarbituric acid (TBA) solution. Absorbance of the sample was measured at a wavelength of 532 nm (T80 UV/VIS Spectrometer; PG Instruments, Ltd.; Lutterworth, UK). A calibration curve was used for the calculation of the results. MDA concentration measurements were carried out on the 1st day, 1st, 2th, 3th month of storage.

Determination of TVB-N

Determination of total volatile basic nitrogen (TVB-N) the concentration of TVB-N in the fish sample was determined by the reference procedure described in the decision of the European Commission 95/149/EC.

Content of TVB-N measurements were carried out on the 1st day, 1st, 2th, 3th month of storage.

Statistical analysis

The obtained data were processed with Microsoft Excel (Microsoft Corporation. (2018). Microsoft Excel. Retrieved from https://office.microsoft.com/excel) and GraphPad Prism 6 software (GraphPad Software, San Diego, USA) using analysis of variance (ANOVA). The results are expressed as the average of three measurements. Results were presented as min, max, mean and standard deviation. Statistical significances between groups were calculated using a t-test of evidence $P \leq 0.05$.

RESULTS AND DISCUSSION

In rainbow trout up to 500g, the lightness (L*) was already after the first month of storage of the card ($P \leq 0.05$) lower than on the first day of measurement. A significant ($P \leq 0.05$) darkening of the meat increased proportionally with the increasing number of months of storage. Redness (a*) had an unproven increasing tendency in the RT group during the 3rd month of cold storage. The yellowness (b*) of the meat was lower in the RT group after the first, second and third month of the certificate ($P \leq 0.05$) than on the first day. In rainbow trout (RT1) up to 3.5 kg, the meat was noticeably ($P \leq 0.05$) lighter (L*) after 3 months of storage. The redness (a*) of the meat had a demonstrably ($P \leq 0.05$) decreasing character, that is, the meat was less red. In yellowness (b*), we recorded a significant ($P \leq 0.05$) decrease in values during all three months of storage compared to the first day of measurement. The results of the first day of analyzes are consistent with the L* parameter of the study **(Wu *et al*., 2021)**, which they reported for the lightness of rainbow trout meat (weighing 1.91±0.38 kg) 46.47±3.99. For parameters a* and b*, the values measured by us in the RT group are lower and in the RT1 group higher than reported **(Wu *et al*., 2021)**. **No *et al.* (1991)** report an increase in all three values of colour parameters, namely an increase in L* from 43.8±1.8 to 51.3±2.4, an increase in a* from 6.1±1.7 and also an increase in b* from 20.6±2.6 to 23.1±26.6, where the fish were analyzed after 3 months of storage at -20 °C. In our experiment, we observed an increase in L* during storage at -18°C, and the values of a* and b* decreased during storage in the RT1 group. In the RT group, there was an unprovable increase in a* and a significant ($P \leq 0.05$) decrease in L* and b*. In the RT group, the meat was red and yellow as in the RT1 group. The content of TVB-N in both of our experimental groups was approximately the same at the beginning and at the end of cold storage after 3 months. On the first day after slaughter, TVB-N in the RT1 group was 5.2±0.361 $mg.100g^{-1}$ and after three months of storage, it increased significantly to 6.01±0.307 $mg.100g^{-1}$. In the RT group, the content of TVB-N after three months (6,000±0,471 $mg.100g^{-1}$) of storage was higher compared to the first day of storage (5,410±0,345 $mg.100g^{-1}$). However, these results are not confirmed by the study of **Mexis *et al*. (2009)** who states already on the first day of storage approximately 10 $mg.100g^{-1}$. Also, the study published by **Rastiani *et al.* (2019)** reported values of TVB-N content at the beginning of storage up to 13.4 $mg100g^{-1}$, which is a much higher value than in our studies. The content of tvb-n was not different during storage in our experimental groups.

We recorded a significant ($P \leq 0.05$) increase in malondialdehyde content after 3 months of storage to a value in the RT group (0.5048±0.3123 $mg.kg^{-1}$) and in the RT1 group (0.641±0.1567 $mg.kg^{-1}$). Malondialdehyde content after 120 days of storage was also noted by **Secci *et al.* (2019)**, who measured up to 2.6 $mg.kg^{-1}$ after 120 days of cold storage (-10) which is a much higher value than here. At the end of the experiment, the content of malondialdehyde was higher in the RT1 fish group than in the RT group.

Table 1 Meat colour of rainbow trout (RT) up to 500g during storage.

	L*				a*				b*			
	1. day	1. month	2. month	3. month	1. day	1. month	2. month	3. month	1. day	1. month	2. month	3. month
Min.	56.26	47.77	51.09	48.83	-1.56	-0.61	-1.34	-1.34	10.33	8.47	7.73	6.53
Mean	59.57^{a}	55.93^{b}	55.93^{b}	54.00^{b}	-0.67	-0.37	-0.32	-0.29	14.82^{a}	11.01^{b}	10.82^{b}	10.65^{b}
Max.	63.96	60.33	61.01	56.49	-0.050	0.14	0.96	0.87	16.82	14.66	13.34	18.42
S.D.	2.73	3.96	2.55	2.45	2.42	1.14	0.96	0.77	2.04	1.60	1.77	3.30

Notes: S.D. (standard deviation); Min. (minimum); Max (maximum); RT (rainbow trout up to 500g); RT1 (rainbow trout up to 3.5 kg); a, b, c = means significant differences between groups (P≤0.05).

Table 2 Meat colour of rainbow trout (RT1) up to 3,5kg during storage.

	L*				a*				b*			
	1. day	1. month	2. month	3. month	1. day	1. month	2. month	3. month	1. day	1. month	2. month	3. month
Min.	44.77	46.06	50.56	49.34	18.91	16.43	14.76	7.7	23.35	17.44	15.06	14.81
Mean	46.66^{c}	49.55^{b}	52.46^{a}	53.31^{a}	20.44^{a}	17.89^{b}	17.58^{b}	11.08^{c}	24.61^{a}	19.39^{b}	18.15^{b}	17.75^{b}
Max.	48.84	54.4	54.34	58.82	23.98	20.12	21.71	14.08	26.49	22.71	22.6	21.25
S.D.	1.41	3.10	1.26	2.94	1.40	1.05	1.74	2.20	0.89	1.61	1.914	1.90

Notes: S.D. (standard deviation); Min. (minimum); Max (maximum); RT (rainbow trout up to 500g); RT1 (rainbow trout up to 3.5 kg) a, b, c = means significant differences between groups (P≤0.05).

Table 3 TVB-N content of rainbow trout during storage (mg.100g^{-1}).

	RT1				RT			
TVB-N	1. day	1. month	2. month	3.month	1. day	1. month	2. month	3.month
Min.	4.60	5.10	5.00	5.60	4.80	5.20	5.20	5.00
Mean	5.20^{a}	5.67^{b}	5.71^{b}	6.01^{b}	5.41^{b}	5.56^{b}	5.63^{ab}	6,00^{a}
Max.	5.80	6.30	6.30	6.50	5.90	5.90	6.40	6.50
S.D.	0.36	0.37	0.45	0.31	0.35	0.23	0.35	0.47

Notes: S.D. (standard deviation); Min. (minimum); Max (maximum); RT (rainbow trout up to 500g); RT1 (rainbow trout up to 3.5 kg) a, b, c = means significant differences between groups (P≤0.05).

Table 4 MDA content of rainbow trout during storage (mg.kg^{-1}).

	RT1				RT			
MDA	1. day	1. month	2. month	3.month	1. day	1. month	2. month	3.month
Min.	0.09	0.26	0.43	0.50	0.10	0.19	0.34	0.20
Mean	0.31^{b}	0.53^{ab}	0.60^{a}	0.64^{a}	0.19^{b}	0.33^{ab}	0.49^{a}	0.50^{a}
Max.	0.69	0.74	0.83	0.88	0.32	0.48	0.75	0.95
S.D.	0.22	0.17	0.13	0.16	0.08	0.10	0.17	0.31

Notes: S.D. (standard deviation); Min. (minimum); Max (maximum); RT (rainbow trout up to 500g); RT1 (rainbow trout up to 3.5 kg) a, b, c = means significant differences between groups (P≤0.05).

CONCLUSION

The aim of our work was to monitor selected degradation products of MDA, TVB-N and colour during cooling of fish for three months after slaughter. Based on the results achieved, we can conclude that even during storage in the freezer (-18°C) fat degradation occurs, but not to the same extent as when stored at a lower temperature (-10°C), which is proven in discussions compared to other studies. Regarding TVB-N, the values we measured after 3 months of storage (-18°C) were lower than those measured in other studies on the first day after slaughter. However, TVB-N values in our experiment increased proportionally in both groups with the length of storage. In the changes of colour parameters, we noted evident differences in individual measured parameters in both experiments, however, the RT group had a different development of colour parameters than the RT1 group. Based on the results achieved and the literature, we can conclude that freezing storage slows down changes and the formation of degradation metabolites, but the storage temperature must be maintained at least -18°C.

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