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OXIDATION DURING NORMAL AND VACUUM PACKED STORAGE OF SMOKED CARP

Bachelor Thesis

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Biological Chemistry

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Annotation

Development of lipid-protein oxidation and firmness in the smoked carp during different storage time at +4°C in normal and vacuum packages was examined.

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SUMMARY

The purpose of this study was to investigate the effect of normal and vacuum package systems on the development of lipid-protein oxidation and firmness of common carp fillet through 3 weeks storage at +4°C.

TBARS, carbonyls and firmness were measured in time 0 and 1 week for samples packed under normal package system and 0, 1, 2 and 3 weeks for samples stored under vacuumed packed system. Results showed significant difference between the fillets stored under different packing conditions. Lipid oxidation by respect to TBARS, indicate no significant difference in amount of TBARS in both package systems. However, protein oxidation showed significantly higher values of carbonyl content in normal packing compared to vacuum packing fillets. Firmness analysis results showed significant increase of the fillet firmness in nrmal packing after 1 week and decrease in the vacuum packing after 3 weeks storage. The results emphasized development of lipid and protein oxidation was more promoted in the carp fillets which were packed under normal storage.

1 INTRODUCTION

Fish has excellent nutritional value, providing high amount of polyunsaturated (PU) n-3 fatty acids (FAs) and high-quality protein. Due to presence of highly unsaturated fatty acids, fish products are prone to quality loss due to lipid and protein oxidation.

Lipid oxidation in foods, such as fish particularly containing PUFAs, has been demonstrated to be connected to the decrease of the quality of the flesh in the period of storage, development of off-flavor components and loss of nutritional value [1]. Increasing peroxides and thiobarbituric acid reactive substances (TBARS) values are the most important signs of oxidation in the fish fillets. Finally, as a result of the lipid oxidation some volatile compounds are generated which are responsible for unpleasant odor during the storage. Photosensitized oxidation, autoxidation, and enzymatic oxidation are three ways for lipid oxidation [1].

Some factors play key roles in the shelf life and maintaining food quality such as: storage temperature, packaging techniques and exist of antioxidants. Lipid oxidation in fish products cause off flavor and odor development which can be slowed down by vacuum packing.

In addition to lipid oxidation, the oxidation of proteins can also cause quality changes in fish. In general, the same factors causing lipid oxidation will also cause protein oxidation. Additionally, the mechanisms and pathways of protein oxidation are similar but more complex than those of lipid oxidation. Radical-mediated oxidation can cause damage to proteins in various ways, such as metal–ion dependent reactions, the autoxidation of lipids and sugars and the initiation by electron leakage [2].

Oxidation consist of numerous pathways which include protein crosslinking, amino acids modifications. However oxidative change in the protein form very broad chemical groups to the proteins, such as aldehyde, ketones [3].

Oxidative modifications of food proteins cause significant changes in texture, affect water holding capacity, nutritional value, solubility loss. Refrigerated storage is from the significant importance in the quality and safety food preservation. Common carp as the type of fatty fish has recommended shelf life of 2 days [4]. [5] presented a shelf life of 9 days for the silver carp stored at 4° C in the vacuum package and 3 days in the normal package storage respectively.

The shelf life of fish fillets is decreased during the storage time by the development of lipid and protein oxidation products. Packaging technique is another way beside the temperature that can influence the shelf life of fish filets.

2 COMMON CARP (*Cyprinus carpio*)

Common carp (*Cyprinus carpio*) is one of the most common freshwater fish. Carp belongs to the freshwater rich family of Cyprinidae. Cyprinidae consist of 20 000 species which may be seen in the water of Asia, Eastern Europe, Africa, Australia. Appearance of the carp is very similar to the goldfish, combination of the colors, white, black red, yellow blue carp may be seen. The body of the carp is composed of two pairs of barbells located at the mouth and forked tail with serrated first spine. Its length may reach up to 120 cm having 60 kg in weight [6].

Carp represents a good source of nutritional components, high protein content and fat and also a source of n-3 polyunsaturated fatty acids and the n-6 polyunsaturated fatty acid. The fatty acids present in the fish meat may reduce risk of high blood pressure, heart disease, stroke and have a positive effect on circulatory and reproductive systems. The fatty acid composition in the carp depends in fed supplementary, it was showed that fillets fed in the pond contained 1.76% fat while the ones fed with additives like wheat had significant level of the fat 11.22%. Also, carp which were fed in pond showed higher content of n-3 and n-6 fatty acids. Also, fish proteins have beneficial effects on human health, monitoring cardiovascular disease, obesity. Nutritional composition of the carp is affected by environmental, food present in the ponds, cultural system of fish production and diet. It was observed during the cultural system of fish production that additives like corn increases fat formation and meat has a juicier appearance [7].

Therefore, it is suggested [7] that lipid content is more associated with the culture system. Carp saves its fat mostly as adipose tissue which contributes to sensory properties, texture and flavor evaluation.

Figure 1 Common carp (*Cyprinus caprio*) [6]

2 LIPID OXIDATION IN THE FISH FILLET

2.1 MECHANISM OF THE LIPID OXIDATION

Fish is a rich source of essential fatty acids and contain high unsaturated fatty acids. Lipids in the fish meat are classified in two classes neutral (NL) or Triglycerides and polar lipids (PL). Main function of the NL is a storage of the energy as the fat, and the PL are involved in the structural function of the cells and metabolic processes happening in there. Lipids are formed from the fatty acids which are esterified with an alcohol. Reactivity of the fatty acids and their prone to oxidation process is described in the levels of n-3 fatty acids (omega 3) which is functionating as the essential fatty acids followed by, n-6 (omega 6) and n-9 (omega 9) fatty acids. N-3 stands for the double bond at the last carbon from the methyl end, while n-6 is the double bond at the sixth carbon from the methyl group and n-9 double bond and the ninth carbon from the methyl group [8].

Mechanism of the lipid oxidation is studied in the three stages,

(1) Initiation, formation of fatty acid radical
(2) Propagation, the free-radical chain mechanism
(3) Termination, formation of nonradical products

Lipid oxidation process consist of the set reactions in which in each step new products are formed. The Figure 2 illustrates the pathways of chain reactions. Thus, initiation reaction starts with the substitution of oxygen from fatty acyl chain to form a free radical which in next step reacts with oxygen to form a peroxyl radical. The propagation of the chain reaction continues with peroxyl radical extraction of hydrogen from hydrocarbon chain withdrawing acyl radical and forming hydroperoxide. Furthermore, hydroperoxide undergoes homolytic cleavage and results in acyloxy and hydroxide radical generation. Breakdown of the already formed products results in the termination process, and saturated unsaturated aldehydes, alcohols, alkanes and alkenes development [10].

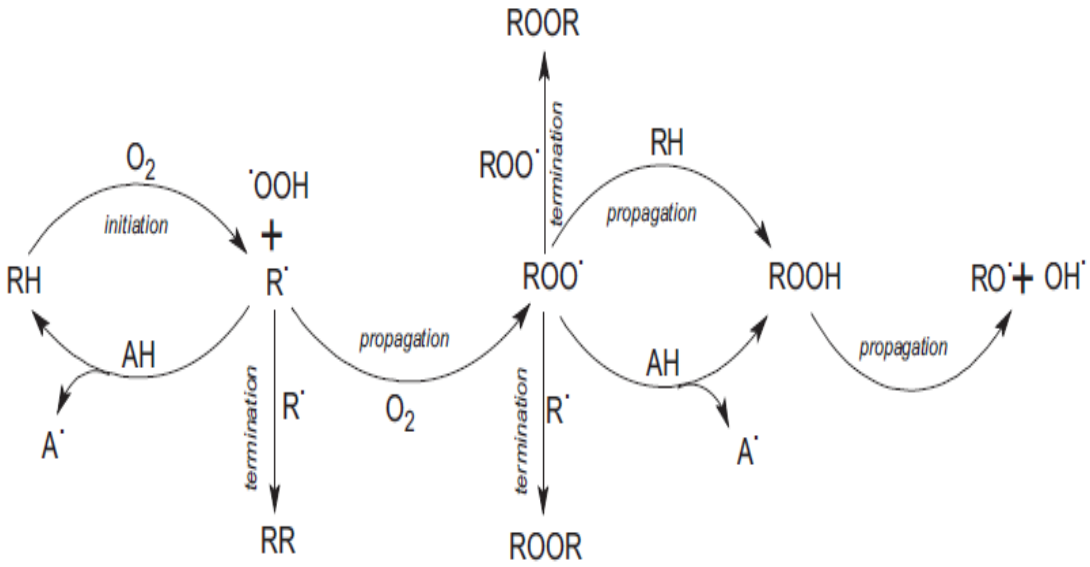

Figure 2 Mechanism of lipid oxidation [10]

2.2 FACTORS AFFECTING LIPID OXIDATION AND FOOD QUALITY

Lipid oxidation is complex process that can generate primary and secondary oxidation products which have impact on the final quality of food products. Primary oxidation products (hydroperoxid), is an unstable compound and converted to the secondary products of lipid oxidation such as, aldehydes, ketones , and alcohol. Development of lipid oxidatin leads to the formation of some compounds which can alter the taste and odor of food muscle products. Lipid together with fatty acids are located in the muscle membranes between muscle fibres and adipose tissue. Fatty acids present there determine nutritional value, sensory quality and color. Also, lipid oxidation promotes protein metmyoglobin which is responsible for the brown color occuring in the meat products [10].

Initiator of lipid oxidation occuring in the fish meat oxygen, light, temperature, transition metals, prooxidants and antioxidant, hemoproteins, cholesterol, enzymes. For example enzymatic activity of lipoxygenases has shown to introuce oxidative changes in the meat structure. Also, cholesterol which is found in the cell membranes promotes oxidation during the long storage, high temperature. Delay of the lipid peroxidation and protection of the cells from formed free radicals is caused by antioxidant which are arranged in two classes, primary and secondary [9]. Primary antioxidants serve to donate hydrogen to alkoxy radicals to block further reaction with another fatty acid. Common antioxidants, are Tocopherols (vitamin E) Ascorbic acid (vitamin C), Carotenoids (vitamin A). Secondary antioxidants are involved in blocking metal catalyzed oxidation, for example EDTA and citric acid [11].

Smoking, salting, and vacuum packaging methods are used to inhibit the influence on lipid-protein oxidation on fish quality and increase the shelf life.

2.3 PRODUCTS OF LIPID OXIDATION

Thiobarbituric Acid Reactive Substances are determined spectrophotometrically employing quantitative measurements of thiobarbituric acid as a marker in lipid peroxidation. Reaction of TBA and malondialdehyde (MDA), which acts as main product in lipid peroxidation is the prime aspect of the method. A recent review summarizes the fundamental advantages of the method precision, sensitivity, and highly reproducibility. Result of the reaction of TBA and MDA, is pink complex which can be studied spectrophotometrically (532-535 nm), chromatographically, or by image processing technique [12].

Malonialdehyde is an aldehyde of three carbon which is formed by various mechanisms. Literature [13] summarizes that only peroxides with alpha or beta unsaturation are efficient in the reactions of cyclization to form a MDA product. In the Figure 3 MDA formation is shown. The mechanism of the MDA formation uses polyunsaturated fatty acid carbon double bond in which the double bond reduces the carbon hydrogen bond posing subtraction of hydrogen by free radical. During the subtraction of hydrogen, the lipid free radical is formed which by oxidation forms peroxyl radical. Formed peroxyl radical reacts with polyunsaturated fatty acids forming a lipid hydroperoxide and free radical. However, the hydroperoxide is not stable it further produces malondialdehyde and 4-hydroxy-2-nonenal. Disadvantage of method is its specificity, during the reaction of the TBA with products such as aldehydes, intermediates are formed which are absorbed spectrophotometrically at 535 nm like the MDA-TBA product. Formation of the MDA-TBA product is dependent on the Iron damage to amino acids, proteins, nucleic acids intermediates [13]. Therefore, in the fatty food MDA may be attached to aldehydes from the sample, react with them and cause not reliable spectrophotometric results. Use of the acid during the analysis of the food enables the MDA content to stay in its free form and successfully determined. Review of MDA determination in traditional fish products suggest [14] that analysis of the fatty food is a complex process as another food components are present and bound to MDA interfering it.

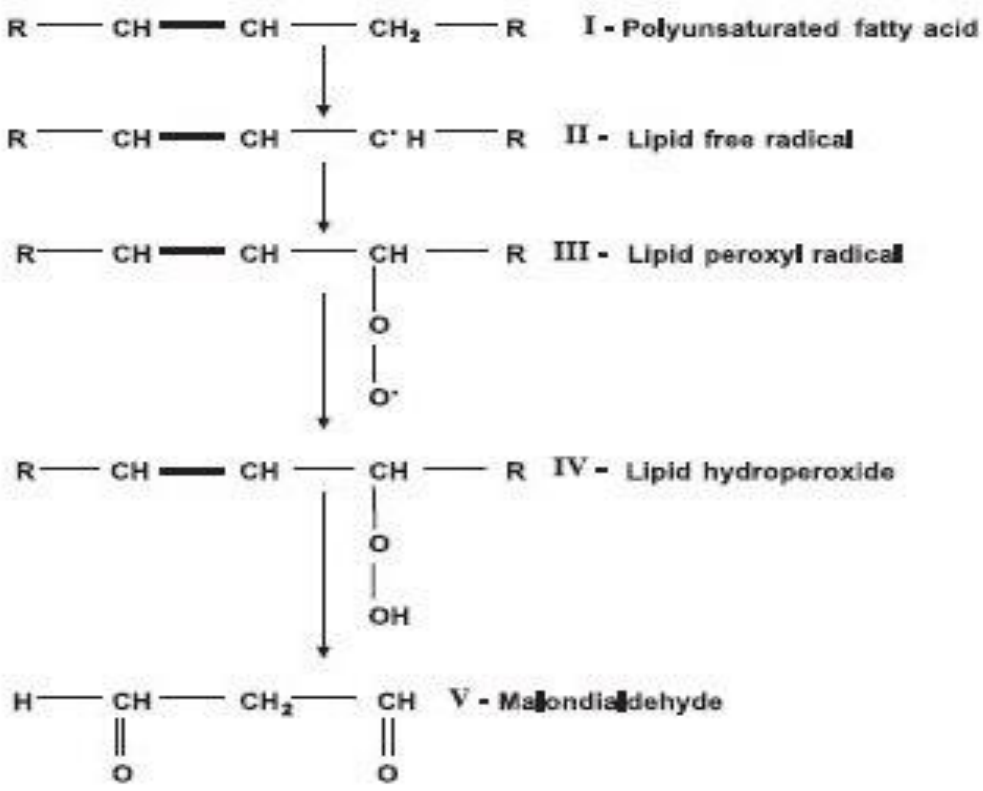

Figure 3 The mechanism of malonialdehyde (MDA) formation [13]

3 PROTEIN OXIDATION

3.1 MECHANISM OF THE PROTEIN OXIDATION

Proteins as the main muscle tissue components are constantly exposed to physical-chemical changes in their structure. These changes are associated with reactive oxygen species (ROS) affecting both lipids and proteins. However, limited information is published about protein oxidations and correlation between protein and lipid oxidation. Nevertheless, changes in the quality, nutritional value, which are caused during a storage method or processing are a target of mechanism of protein oxidation. Reactive oxygen species (ROS) may catalyse oxidative reactions and cause directly protein oxidation, or as the result of lipid oxidation products such as malonaldehyde, which interacts with protein resulting in protein structure alteration [15].

Oxidation of the food proteins as explained in the study of [16] lead to change in the protein function. In the fish meat oxidation is correlated to the loss of solubility, texture changes and water holding capacity. Two processes which determine course of oxidative changes are the amino acid oxidation causing formation of protein crosslinked products, thus, thiol groups oxidation, carbonyls and hydroperoxides formation, and change in protein backbone which results in the fragmentation, aggregation of proteins [9].
On another hand, oxidation in the side chains of amino acids especially, lysine, arginine, tyrosine, histidine which are the most affected in the oxidative changes can form carbonyl groups that eventually result in a textural and solubility redocument modifications [16].

The mechanism of the protein oxidation compromise of larger variation of oxidation reaction and products than in the lipid oxidation. To determine protein oxidation several methods are employed, detection of carbonyl groups, sulphydryl groups (thiol content) or formation of dityrosine. Detailed monitoring of the mechanism of protein oxidation can be employed using various methods of mass spectrometry, electron spin resonance and high-pressure liquid chromatography together with fluorescence spectroscopy [11].
As illustrated in the Figure 4 protein oxidation is initiated when a hydrogen atom is abstracted from the protein to generate a carbon-centered radical (C•) and in the presence of oxygen is converted to an alkylperoxy radical (COO•). The following reaction of (COO•) with hydrogen atom abstraction from another molecule leads to alkyl peroxide (COOH) formation. Subsequent reactions lead to the formation of the alkoxy radical (CO•) and hydroxyl compounds (COH). In addition, two carbon-centered radicals can react with each other in the absence of oxygen, to generate carbon–carbon cross-linked derivatives [17].
The oxidative changes may occur on the amino acid residues thus oxidizing thiol groups, hydroperoxide, carbonyl formation and the protein backbone causing changes in the polypeptide chain, fragmentation, and aggregation of proteins. Effect of the protein oxidation on amino acids, cysteine, lead to formation of sulfenic acid, sulfnic acid or disulfide bonds [16].

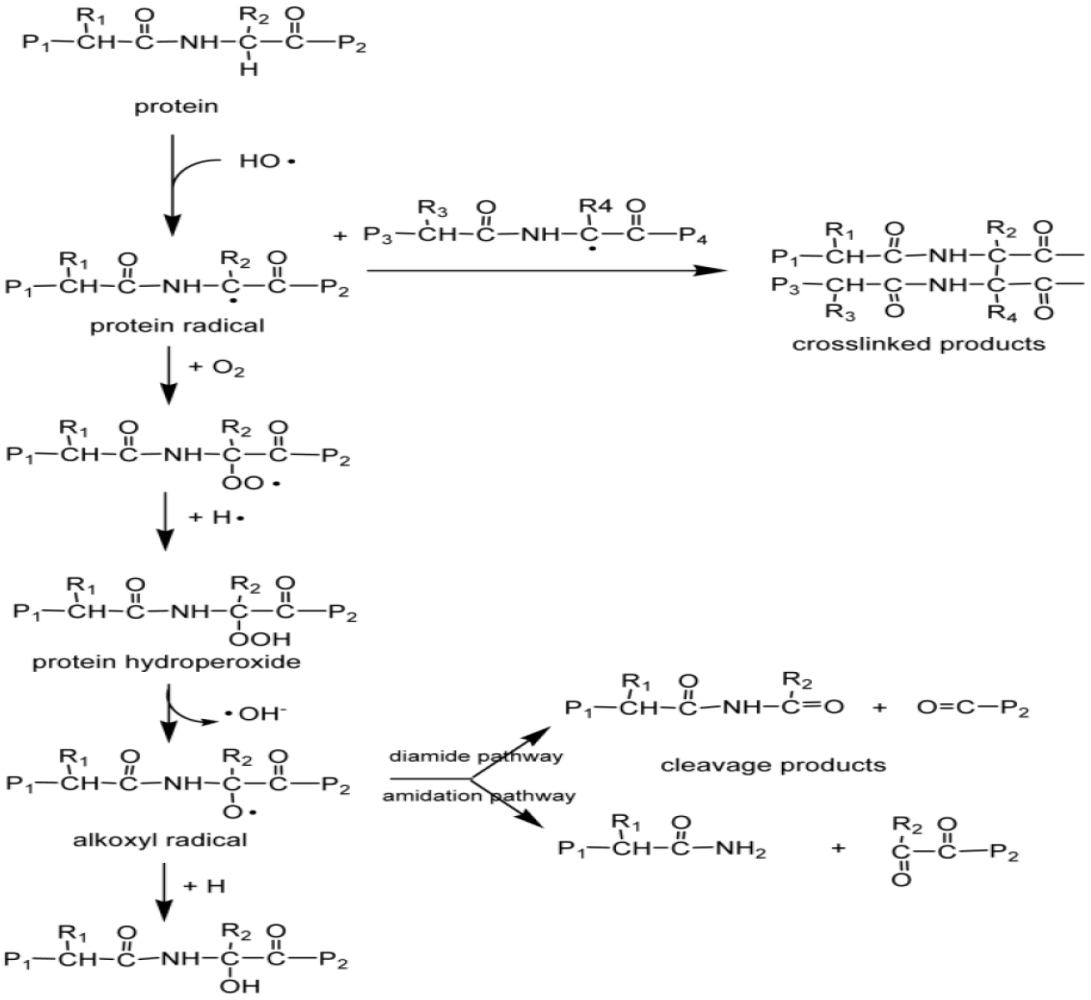

Figure 4 Protein oxidation by hydroxyl radical, formation of cross linked products [18]

3.2 EFFECT OF PROTEIN OXIDATIONON ON FISH QUALITY

Physical and chemical properties of the amino acids, processing treatments, storage conditions contribute to change in the oxidation in foods. Primary, secondary, tertiary protein structures are affected too. Due to presence of polar, nonpolar, charged protein groups on the surface of protein it may alter its function and promoting the oxidation. Instability of those protein structures may be associated with formation of disulphide bonds with reactive groups of Cysteine (Cys) residue [19]. Texture properties of the fish undergo changes during protein oxidation. Enzymes μ calpin and m calpin which consist of cysteine and histidine residues may be inactivated by the oxidation which has the effect on the reducing water holding capacity.

Also, SH- containing amino acids residues are prone to oxidation as the thiol groups form thiyl peroxy radicals, or the formation of sulphur acids, for example unstable sulfenic acid and sulfonic acid. These reactions lead to protein aggregation, loss of solubility, off - odor. Another hypothesis for the firmness, in the connection with the protein oxidation is formation of protein disulphide cross- linking on the myosin heavy chain (MHC) which decreases tenderness during the storage time. Lipid oxidation can be affected by the presence of the protein radicals, peroxynitirite anion is susceptible for the meat spoilage, introducing colour change from red to brown and flavour degradation. In meat lipid oxidation development is associated with the presence of myoglobin [9].

Metmyoglobin have prooxidant activity, which promote oxomyoglobin oxidation [20]. This actvity was affected from secondary products of lipid oxidation, aldehydes. Thus, change in the meat color is related to the amount of the metmyoglobin,oxymyoglobin. Metmyoglobin is the initiator in the chain reaction and therefore aldehydes effect myoglobin which develop oxomyoglobin oxidation. The discoloration of the meat changes to the brown pigment as the consequence of the heme- protein oxidation.

Changes in the texture are also altered by the protein in a fish, myosin. Due to protein oxidation myosin affects myofibrillar protein structure thus, influencing the texture change by increasing the protein carbonyls level with storage time increase [11].

3.3 EFFECT OF PROTEIN OXIDATION ON FIRMNESS

Fish quality, appearance, taste, are the factors involved in firmness determination. Fish are processed by different packing methods to maintain their firmness for the various industry supply. Different components play a role in fish firmness measurements, physicochemical processes, protein degradation, decomposition of the ATP, which destroys muscle of the fish and show quality degradation. Firmness is closely related to the internal factors of the structure of connective tissue of the fish. Also, it represents crucial parameter in determination of the fish freshness. Figure 5 illustrates the key fragments of connective tissue of muscle cells, myotomes, which are made up of single muscle fibers located parallel and coupled with connective tissue forming myocommata. However, it has been shown that the most consistent protein is the collagen regulating the fillet stability. Thus, accounts from 3% to 10% of protein [21].

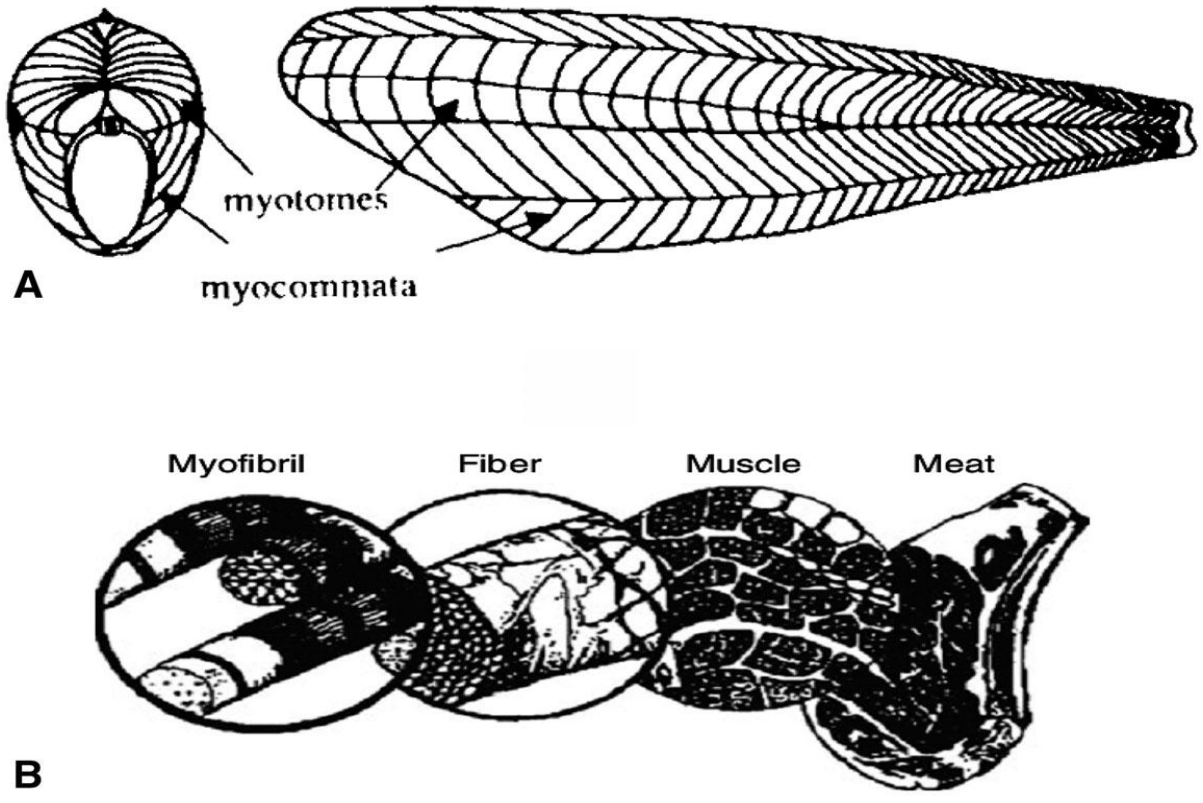

Figure 5 Connective tissue of the muscle cell, myotomes, myocommata [21]

4 PACKING TECHNIQUES

4.1 INTRODUCTION OF DIFFERENT PACKAGING METHODS AND EFFECTS ON THE OXIDATION PROGRESS OF LIPID AND PROTEIN

In addition to packaging, smoking is also a method used in fish processing to lower the oxidative changes and rancidity. Shelf life of the smoked meat products depends on the several factors including oxygen levels during storage, temperature, presence of the bacteria in the raw products, decrease in the water activity. Preliminary process of smoking technique is the salting, which has a role to eliminate the water content thus as result reduce the spoilage, oxidation, and significantly increase the shelf life of the fish [30]. Lipid oxidation in the meat is promoted by the acceleration of the metmyoglobin and protein deterioration. Storage conditions, oxygen concentration, light are the factors involved in oxidative reactions. Additionally, the protein oxidation has shown to negatively influence texture and water holding capacity [22].

Consumption of fish stored in aerobic conditions reveal the increase in the pH value, water activity, amino acids which are substrate for the oxidation and spoilage. Product stored in the aerobic condition have limited shelf life compared to vacuum products. Also, in aerobic conditions skin surface of the fish is the first to enhance the spoilage due to evolution of bacterial colonies on it [22]. In the modified atmosphere packing as explained in the literature, mixture of gasses carbon dioxide, oxygen, nitrogen are used in order to maintain shelf life and meat quality. Carbon dioxide serves to prevent bacterial growth, oxygen influences the flavor and meat color, while the nitrogen gas act as neutral gas and does not affect meat color and flavor. Key facts reveal that highest effect of the carbon dioxide is a 0°C and at 5°C carbon has lower effect. Modified atmosphere packaging shows disadvantage in the shorter shelf life compared to vacuum and for product distribution it requires bigger space. Vacuum packing process involve the removal of air from package in which food is stored. Upon the decrease of the oxygen concentration in the package, microbial activity and spoilage of the bacteria can be inhibited [23]. Color of the spoiled meat is purple myoglobin and changes during the storage time to greyish- brown metmyoglobin. Crucial factor which affects meat spoilage is pH value which upon increase results in spoilage [24]. Also, smaller volume in vacuum package shows the advantage in the easier distribution and processing [25].

5 AIM OF THE STUDY

The purpose of this study was

1. The evaluation of lipid and protein oxidation influenced by different packaging systems
2. Effects of lipid-protein oxidation on firmness in normal and vacuum packed fish fillet

6 MATERIALS AND METHODS

6.1 EXPERIMENTAL DESIGN

21 fresh smoked common carp fillets were supplied from the local market. Fish fillets were immediately packed in two different packaging system (normal and vacuumed) and stored in the refrigerator at +4°C for 1 and 3 weeks respectively. For each time point 6 fillets were used in duplicates. Firmness analysis was performed on the dorsal part of fillets (n=6) for each time point. Moreover, to determine the oxidative changes in the fillets (n=6) for 3 weeks storage under vacuumed pack and 1 week storage under normal pack systems. TBARS as a sign of lipid oxidation and carbonyl content as a sign of protein oxidation were investigated.

6.2 CHEMICALS AND REAGENTS

2-Thiobarbituric acid and 1,1,3,3-tetramethoxypropane, trichloroacetic acid, methanol, butylated hydroxytoluene, choloridric acid, guanidine, 2,4-dinitrophenylhydrazine, ethyl acetate and sodium hydroxide were purchased from (Sigma Aldrich, Steinheim, Germany). Phosphoric acid, potassium chloride were obtained from (Lochner, Prague Czech Republic). Ethanol and hexane were procured from (Penta s.r.o. Prague, Czech Republic)

6.3 LIPID OXIDATION

TBARS method was carried out according to the (Miller 1998). Samples were left for short time to thaw, fat was removed and approximately 1 g of muscle tissue was used for extraction. To the samples 9.1 mL of 0.61 mol L^{-1} trichloroacetic acid (TCA) and 0.2 mL of 0.09 mol L^{-1} butylatedhydroxytoluene (BHT) in methanol were added. Suspension was homogenized using an UltraTurrax (Janke & Kunkel, Staufen, Germany, T25IKA-Labortechnik,) for 3 × 20 s with a speed of 14000 rpm. After the suspension was homogenized it was filtrated. 1.5 mL of the filtrate was transferred two times to the new tubes. 1.5 mL of the TBA was added to the first tube, test sample, and 1.5 mL of the water was added to the second tube serving as a blank

sample. The tubes were left in the darkness overnight at the room temperature 20° C. The absorbance of the samples was detected at wavelength of 530 nm against blank sample of water using a UV -visual spectrophotometer (Specord 210; Analytik Jena, Germany). The results were expressed as malonaldehyde equivalents (MDA) in μg/g in duplicates. [26]

6.4 PROTEIN OXIDATION

The protein oxidation, carbonyls determination, was performed by the method of (Olivier et al. 1987). 1 gram of the fish fillet was mixed with the 10 mL of KCl (0.15M) and afterwards homogenized using UltraTurrax (Janke & Kunkel, Staufen, Germany, T25IKA-Labortechnik,) for 3 × 20s at a speed of approximately 14000 rpm. Duplicates of the 100 μl of the solution were transferred to the 2 mL Eppendorf tubes. 1 mL of 10% TCA was added to each tube ad centrifuged at 5000 rpm for 5 min. After centrifugation supernatant was removed. 1 mL of the HCl (2N) was added to the blank tube and the 1 mL of the DNPH to another tube. Samples were left to stand at ambient temperature for one hour and after every 20 min intervals tubes were shaken and vortexed every 20 min. 1 mL of the TCA (10%) was added to each tubes and centrifuged for the 5 min at the 5000 rpm, afterwards supernatant was removed. 1 mL ethanol-ethyl acetate (1:1) was added to the pellets and centrifuged for 5 minutes at 10000 rpm. Two washing steps of the pellets with ethanol-ethyl acetate (1:1) were carried out. 1.5 mL of guanidine solution (6M) was used to dissolve precipitated protein and it was afterwards centrifuged for 2 minutes at 5000 rpm. Supernatant was read in the spectrophotometer at 280 nm and 370 nm to proteins and carbonyls. At the 280 nm the protein concentration was measured and determined using standard of bovine serum albumin, and at the 370 nm carbonyl concentration (DNPH) was calculated based on extinction molar coefficient, hydrazine (21.0 mM^{-1} cm^{-1}). [27]

6.5 FIRMNESS ANALYSIS

Firmness was defined as the maximum force detected during first compression, expressed in newton (N). The firmness analysis of the fillets was done using instrument (TA-XT. Plus, Stable Micro systems UK). The fillet muscle fibers were mashed by end of the cylinder (10 mm diameter, type P/10) with the speed of 2mm s^{-1} until compression of 50% its initial thickness.

6.6 STATISTICAL ANALYSIS

The mean values and standard deviation were statistically evaluated by processing six values for each packaging system. The level of significance between chemical parameters and firmness in two packaging conditions were calculated by using one-factor ANOVA analysis in the Statistica CZ 12 software package. Significant differences were in both cases considered at $P<0.05$.

7 RESULTS

Protein oxidation

Results of the carbonyl content in two different packaging (normal and vacuum) methods for 3 weeks storage at +4°C were shown in Figure 6. The carbonyl content under normal packaging system increased significantly ($p<0.05$) from 2.48 to 3.27 (nmol/mg) after 1 week storage at +4°C. On the other hand, fillets which were kept under the vacuum package condition showed a slight decrease in the amount of carbonyl content after 1 week then, increased significantly ($p<0.05$) up to 3.09 (nmol/mg) after 3 weeks storage at +4°C.

Furthermore, time to time comparison between normal and vacuum package fillets showed that, after one week the amount of carbonyls content in the normal packed fillets were significantly ($p<0.05$) higher than vacuumed packed fillets.

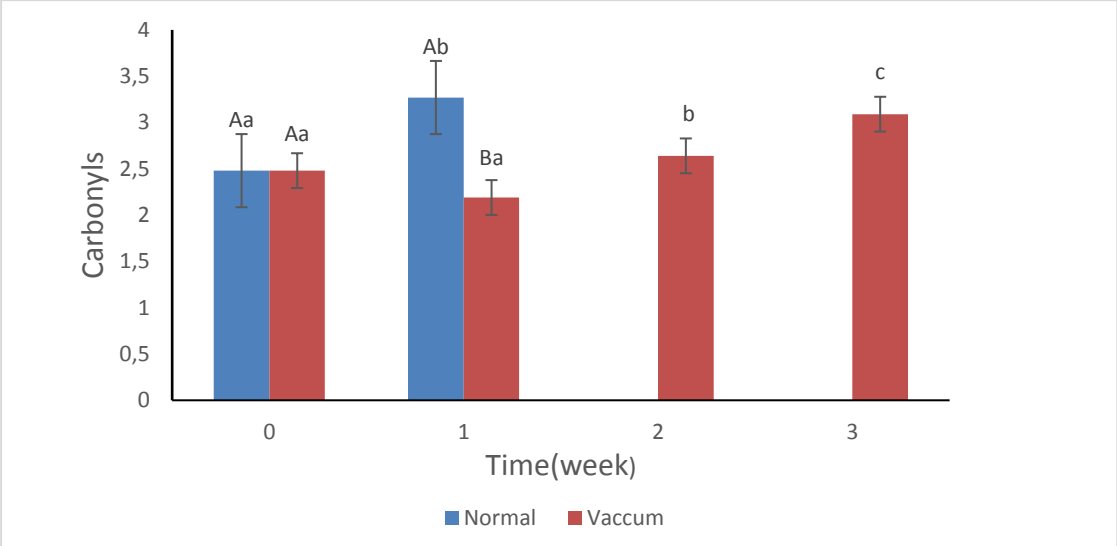

Figure 6 different capital superscript letters in a column bar indicate significant differences ($p<0.05$) within each packaging system. Small superscript letters in a row indicate significant difference ($p<0.05$) between each packaging systems at the same time point.

Lipid oxidation

Results obtained in the TBARS determination in normal and vacuum packaging methods during the storage time of 3 weeks at +4°C are shown in the Figure 7. The TBARS content in the normal packaging system slightly increased ($p<0.05$) from 0.29 to 0.3 nmol/ml in the storage week 1. Vacuum package revealed relatively constant values in the TBARS content after storage week 1 then, minor decreased to 0.28 nmol/mL, in the storage week 2. TBARS value during the week 3 show increase ($p<0.05$) up to 0.31nmol /ml.

Despite the two diverse packing techniques TBARS values did not change significantly during the storage time.

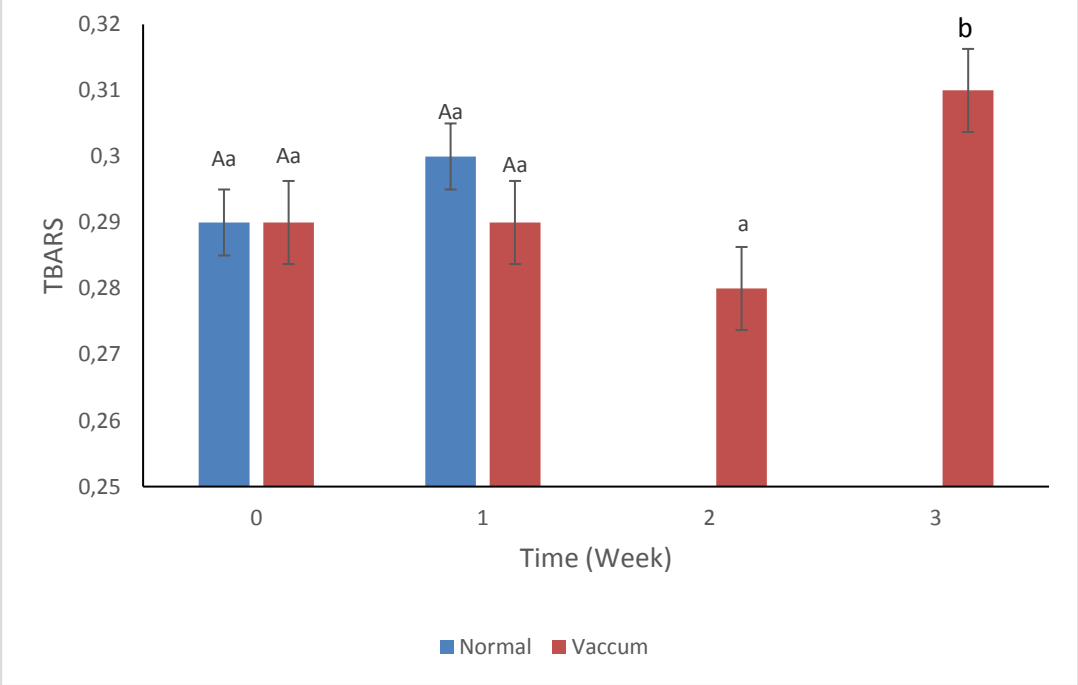

Figure 7 each packaging system are indicated with different capital superscript in a column bar showing the significant differences of ($p<0.05$). Significant difference ($p<0.05$) between each packaging systems at the same time point are represented by small superscript letters in row.

Firmness

Firmness determination in vacuum and normal packing is shown in the Figure 8 with the three weeks of storage time at +4°C. Fillets which packed under normal pack showed a significant increase in the firmness ($p<0.05$) from the 674 N to the 732 N during 1 week storage. Fillets which packed under vacuumed pack showed a significant increase in the firmness ($p<0.05$) from 674 N to 816 N until the second week then it decreased significantly to 641 N after 3 weeks storage.

Comparing between both packaging systems did not show any differences after 1 week storage at +4°C.

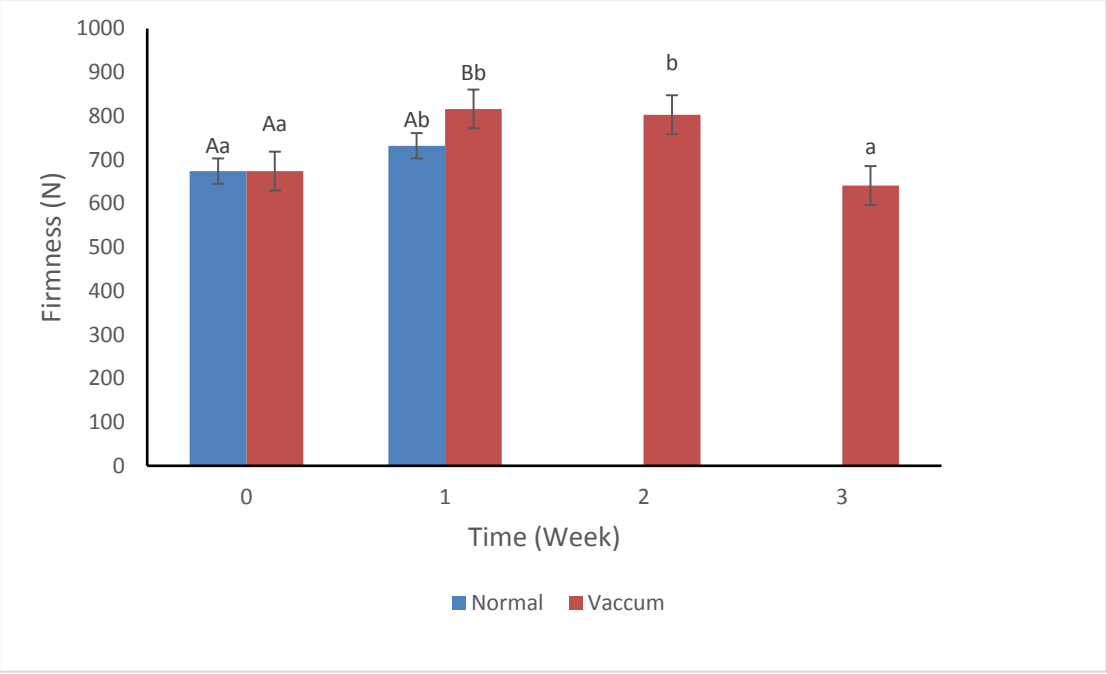

Figure 8 Different capital superscript in a column bar represent vacuum and normal packaging system significantly differing of ($p<0.05$). Small superscript letters in row demonstrate significant difference of ($p<0.05$) at the same time point in vacuum and normal packaging.

8 DISCUSSION

Higher amount of TBARS in the fillets that were stored in the normal package rather than vacuumed package observed in our study. [28] reported the decrease in TBARS content in vacuum packed of common carp during 15 days of storage at 5°C influenced by addition of antioxidants. Specially, the normal packed carp fillets showed significant higher TBARS value due to storage period progression. [29] reported that refrigerator storage 4°C of the three carp burgers, control sample, 5 gm burger-ginger mix, and 10 gm burger-ginger mix, showed increase in the TBARS value in the 19 days of storage.

In a contrary to our results, [30] revealed significant increase of TBARS content in the vacuum and modified atmosphere package of liquid smoked rainbow trout content during the storage time of 120 days at 4°C. They reported high increase in the TBARS content in vacuum packages (10.45, 8.36 MDA/kg) and significant lower value of (7.22and 6.36 MDA/kg) in modified atmosphere packages respectively. Also, they suggested that consumption limit, is 7-8 MDA/kg showing the quality loss in the samples.

In line with our results, finding of [31] on the effects of UV-C radiation on the rainbow trout fillets stored in aerobic packing, vacuum, and modified atmosphere packing, during 22 storage days at 4°C. Results reported increase in the TBARS content in aerobic packages as well as modified atmosphere packages on the 9^{th} storage day. In the vacuum technique increase in the TBARS was detected on the 16^{th} storage day.

Fish fillets that packed in the normal packages, were exposed to the oxygen which activate the pro oxidants causing the shelf life decrease, by the MDA content increase. Regarding the TBARS properties, we observed higher amount of TBARS in fillets from normal packages during 1 week storage compared to 1 week stored fillets in the vacuumed pack, this might be related to the presence of oxygen which could form the free radical chains, thus, forming fatty acid hydroperoxides, which further initiate lipid oxidation. The 2 week storage of vacuum package represented lower value of TBARS which indicates that storage time was not long enough to produce secondary lipid oxidation products. However, 2 week storage time in vacuum may preserve the nutritional quality of fish fillet. Main components of lipoproteins, linoleic acid and arachidonic, are present in the meat products. These acids during mincing, packing and storage may promote lipid oxidation and negatively influence off odor, flavor, color change. Also, fish muscle tissue is prone to the changes caused by transition metals, like

Iron. Possible release of heme Iron in the 3 week vacuum storage promoted the lipid oxidation. Additionally, promoted lipid oxidation can also influence proteins, by the loos of protein solubility, denaturation and crosslinking formation. Favorable conditions for lipid peroxidation and meat muscle denaturation is related to myoglobin presence, ranking the flavor and brown color. Unsaturated fatty acids can be rapidly oxidized and influence the meat color. Firstly, oxidized are unsaturated fatty acyl groups from muscle fiber membrane.

Significant increase in the carbonyl content of normal and vacuum packed, indicates the protein degradation during the storage time. A study [32] showed that carbonyl concentration in the silver carp fillets during 72 h of postmortem stored at 4± 1°C increased in the period of storage time. [33] studied protein oxidation of horse mackerel fillets that stored in the plastic bags under vacuum condition for 96h at 5°C and reported that protein oxidation development during the storage time. It means that the carbonyl concentration increased significantly after 12h of storage and was constant until the end of storage time.

[24] reported lower carbonyls content in the vacuum packed of Korean native cattle stored at 4°C and they observed no notable change in carbonyl content in the modified atmosphere packages during the storage of 10 days and 14 days. In contrary to our study, [34] investigated protein oxidation in chicken breast meat kept in oxygen permeable bags at 4°C for 7 days indicating no significant increase in carbonyl content during 7 days of storage period.

Thus, results showed that proteins were oxidized during the storage in both normal and vacuum packages. Protein oxidation has negative influence on the water capacity and texture and therefore during the storages oxidative stability influenced packages. In the first week of the storage in normal packages higher carbonyls content may be associated with protein oxidation progression, thus oxidative changes are occurring regardless of packing technique. On another hand in the vacuum packages at the same storage time the carbonyl content decreased significantly. This is due to vacuum package which is effective method for inhibition of oxidation.

Oxidative changes which are occuring during the storage in the 2 week and 3 week in vacuum packs may be affected with the carp feed, preparation, smoking and salting procedures. As such, meat treated with the NaCl may cause formation of hypervalent ferrylmyoglobin and thus generate protein oxidation and meat discoloration. Also, due to salting effect in the fish muscle

distruption of the membranee may occur thus leading to oxidative changes in proteins caused by catalyst -lipid-protein interaction. Nevertheless, storage of the three weeks have influenced salt soluble myofibrillar proteins which negatively influence the structural changes and promote pro oxidant factors. Therefore, 3 weeks vacuum storage at +4°C revealed protein carbonylation.

In the fish fillets stored in normal and vacuum packaging higher concentration of proteins and their proximity to hydroperoxides, hydrogen peroxide, detected oxidative changes in the proteins. Additionally, [35] revealed that proteins have ability to be modified independently, by the reactive oxygen species, or by hydrogen peroxide and hydroperoxides. Thus, process of oxidation is dependent on the storage time, packaging method, temperature. [31]

In line with our results, [36] investigated textural properties (hardness, cohesiveness, springiness, gumminess, chewiness), of atlantic salmon treated with two smoke flavors during 45 days of storage time at 2°C in oxygen resistant bags and found that during the storage time textural parameters were increased in treated samples and a control sample showed decrease in the all parameters. They correlate that smoking effect had an influence on the textural and pH changes. It increased the activity of the enzymes in the muscle tissue which affect the proteins and cause breakage of connective tissue. [37] studied changes in texture of liquid smoked silver carp stored in the aluminum foil in the period of 24 days. Also, [38] reported a significant reduction in the texture and water holding capacity (WHC) of bighead carp (*Aristichthys nobilis*) at the +4 and -3°C. [39] investigated air, vacuum, and modified air packages of smoked sea bass fillets that stored at 4°C for 42 days and reported a decrease in color and texture in the fillets that under vacuum package, while the samples which packed in the air packages showed WHC was reduced during the storage time. Also, smoking technique increased water capacity content, and decreased pH value.

Regarding to the firmness results, we observed a significant increase in the firmness of fillets from both packages during 1 week storage. Normal package sample during the 1 week storage showed decrease in comparison to the vacuum, this can be associated to the formation of disulfides on the SH- side chain consisting of amino acids residues affecting the cross linking of the connective tissue which at the end increased degradation rate of the fillets. Vacuum package after 1 week storage showed higher firmness compared to 1 week normal package

storage which may be due to functionality of vacuum package to terminate development of peroxides products affecting both texture and rancidity. Furthermore, increasing in the firmness is associated with enhanced protein carbonylation and the cross-linking that show the dominant in the modifications of meat products. During the 2 weeks storage in the vacuum packages firmness decreased compare to the time 0 which is influenced by enzymatic and oxidative protein degradation. Storage time, salting, and smoking, can cause activation of the reactive substances which as a product have lipid radical further altering the oxidative damage. The technique of smoking has the influence on the muscle structure. It may result in the reduction of the fiber diameter and extension of the intercellular space in the cells as the storage time increases.

The decreasing of firmness over storage time could be caused by increased oxidation and the destruction of myofibers from myocommata. [40] reported that, oxidative reactions in meat products are the key factors of texture, nutritive, flavor losses, due to generation of toxic compounds that influence the meat muscle functionality.

9 CONCLUSION

This study illustrated that development of lipid-protein oxidation and firmness changes in the fish fillets which were stored at +4°C in the vacuum package were slower than fish fillets stored at +4°C in the normal package. It can be concluded that normal package has strong effects on the liability of fillets to lipid- protein oxidative reactions. Also, to maintain the quality and freshness the refrigerated storage at +4°C showed to be effective in short- time product storage.

As a result, the vacuum package slow down the chemical deterioration, and extended the shelf life of the carp fillets up to 2 weeks of storage at +4°C.

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