INFLUENCE OF DIFFERENT TYPES OF MODIFIED ATMOSPHERE PACKAGING ON PHYSICOCHEMICAL PROPERTIES OF COLD-SMOKED SALMON CURED WITH SODIUM AND POTASSIUM CHLORIDE

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Abstract
The aim of this study was to determine the influence of modified atmospheric composition on physicochemical parameters of cold-smoked salmon fillets. Salmon fillets were cured with sodium chloride (NaCl) and potassium chloride (KCl) and packaged in the modified atmosphere: vacuum, 100% CO_2, 50% CO_2, 50% N_2 and 30% CO_2: 70% N_2. The study was conducted for four weeks. The mixtures in the modified atmosphere packaging were found to have an effect on the physicochemical properties of salmon. Higher values of salmon fillet hardness (29.35 and 29.69 N) were characteristic to products made using KCl for salting and packing with 50% CO_2: 50% N_2 and 30% CO_2: 70% N_2, respectively. When sensory products were evaluated (the study was performed only in the first week of storage), the products salted with KCl were seven times bitterer in comparison with the products salted with NaCl.
Oxidative stability was assessed by the accumulation of peroxides, the formation of free fatty acids, and the reaction of the formed oxidation products with 2-thiobarbituric acid (TBA). The obtained results indicated that the least peroxides were formed in vacuum-packed cold-smoked salmon samples and salted with KCl (0.05 kb / 100 g), whereas the CO_2 gas present in the package promoted the formation of these compounds. NaCl is a more effective tool of maintaining the oxidation stability of fats in terms of the formation of free fatty acids. The effect of different gases on the formation of free fatty acids was not observed. The secondary oxidation processes are most effectively inhibited by vacuum, as the least amount of secondary oxidation products during storage (absorption 0.23-0.33) was present in the samples.

Keywords: salmon, modified atmosphere, sodium chloride, potassium chloride, peroxide value, free fatty acids, TBA.

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INTRODUCTION

Fish has been a staple food for several centuries in many countries. The major advantage of fish is that it has a high nutritional value and is easily digestible.

However, fish belongs to the group of highly-perishable products. Various canning and packaging methods are used to extend the shelf life of fish and fish products. Products packaged in an air medium are affected by three main adverse factors: oxidation, bacterial and fungal growth. MAP (Modified Atmosphere Packaging) is a packaging system that uses a combination of different gases for the food industry, in particular common mixtures of carbon dioxide (carbon dioxide E290), nitrogen (E941) and oxygen (E948) ((EU) 1129/2011). The gas content of each component is determined by the technological processes and safety of the food. In addition, the effect of each gas is different on the food. Carbon dioxide actively protects against the growth of bacteria and fungi. Oxygen causes fat oxidation and promotes the development of aerobic bacteria and fungi, but creates favourable conditions, e.g. preservation of red meat colour and inhibit the proliferation of anaerobic bacteria (Thippareddi et al., 2010; Brody et al., 2008). The walls of the MAP packaging can break down in the presence of a high concentration of carbon dioxide in the closed environment. As carbon dioxide dissolves in fish tissues, the contents of the package may be damaged. To avoid damage of the package, nitrogen is used as a balancing gas, which prevents the package from shrinking. Carbon dioxide and nitrogen are mixed in proportions depending on the composition of the product (Dondero et al., 2004). Typically, fresh fish is packaged in an atmosphere of all three gases and the following ratios are commonly used: N_2: CO_2: O_2 – (0 – 30): (30 – 70): (30 – 40) %. The most common ratios applied for

packing oily fish without oxygen gas: N_2: CO_2 – (30 – 40): (60 -70) %. Droplets may form inside the package. This occurs when the packaging contains too much carbon dioxide. This problem can be solved by selecting the appropriate gas mixture and placing absorbent paper under the fish.

Salt (NaCl) is commonly used in fish processing as a preservative and flavouring additive. Salt helps to extend shelf life and has a significant effect on water retention, fat binding, colour, taste and texture. However, sodium intake is a major problem in modern society, as it raises blood pressure and promotes the development of cardiovascular and kidney diseases. For these reasons, it was decided to partially replace the NaCl salt with KCl. Both salts have similar properties, but potassium chloride consumption is not associated with the development of hypertension and cardiovascular disease (Pohl et al., 2013). However, the use of KCl is mainly limited by its bitter and astringent taste in the mouth. The process of preserving fish using salt takes place due to the synergistic action of salt – absorption, protective effect of smoke compounds and dehydration. However, the goal is not only to slow bacterial growth and enzyme activity, but also to soften or alter the taste, texture and structure of the raw material, developing a product with a characteristic taste and a longer but limited shelf life (Müller et al., 1998).

In the process of hydrolysis in fish fat, free fatty acids are formed, which are unstable and oxidize rapidly. Fish fat is characterized by higher levels of polyunsaturated fatty acids, which are less resistant to oxidation, resulting in the formation of hydroperoxides and other, often toxic, secondary oxidation products during storage. Peroxide formation (measured by peroxide value) is considered an indicator of primary oxidation, and the value of 2-thiobarbituric acid (TBA) is an indicator of secondary oxidation (Ježek et al. 2010). Changes in fish fat lead to a deterioration in quality during long-term storage, especially under inappropriate conditions. Lipolysis, oxidation, and the products formed during these processes interact with other fish components (Babic et al. 2014).

The aim of the study is to determine the influence of the modified atmospheric composition on physicochemical properties of cold-smoked salmon cured with sodium and potassium chloride.

MATERIAL AND METHODS

Preparation of raw materials

Salmon is gutted, fins and head are removed. Salmon is filleted and sprinkled with salt (3% salt is used per 100 kg of fish). Salmon fillets are cured with vacuum-treated salt (NaCl) and these samples are labelled LN, and the other fillet is salted with KCl and the samples are labelled LK. The salmon is cured for 24 hours at a temperature of 0-6 ^{0}C. After salting, the salmon fillet is rinsed, air-dried and smoked in cold smoke (24 - 28 ^{0}C) for up to 3 hours. The smoked salmon fillet is cooled to 0-6 ^{0}C, cut into pieces and packed in vacuum and modified atmosphere packages. Samples packaged in vacuum are labelled LN1 and LK1, packaged in a modified atmosphere: 100 % CO_2 – LN2 and LK2, 50 % CO_2 and 50 % N_2 – LN3 and LK3, 30% CO_2 and 70% N_2 – LN4 and LK4. Samples were stored at 0-6 ^{0}C for four weeks carrying out a weekly analysis.

Methods of the analysis

Moisture content

Moisture content of the samples was measured in compliance with the Lithuanian Standard Board standards LST 1614:2000. Approximately a 3-5 g sample was placed in the previously dried and weighed boxes. The sample was then dried in a hot air oven at a temperature of 103±1^{0}C until a constant weight was obtained. The sample was analysed in triplicates and the mean was recorded. The percent moisture content was calculated as follows:

$$Moisture\ content\ (\%) = \frac{(w_2 - w_3)}{(w_2 - w_1)} * 100$$

W_1 – weight of the container with a lid; w_2 – weight of the container with a lid and the sample prior drying; w_3 - weight of the container with a lid and the sample after drying.

Free fatty acids content

Standard method of AOAC (2001) was used to determine free fatty acids. A 10 g ground sample of cold-smoked salmon was placed in the flask. 50 ml benzene was poured and kept for 30 min for extraction of free fatty acids. 5 ml benzene, 10 ml alcohol and phenolphthalein as indicator were added and titrated against 0,02 N KOH till light pink colour occurred and remained for 15s. Percentage free fatty acid was expressed as oleic acid. The FFA analyses were performed in triplicates.

Peroxide value

Measurement of peroxide number was carried out by the method described in AOCS (2016). Chloroform, glacial acetic acid and freshly prepared saturated potassium iodide (KJ) solution are added to the weighed sample. The mixture is stirred very well and kept in a dark place. Distilled water is then poured into the flask, starch solution is dripped and the released iodine is titrated with sodium thiosulphate solution until the blue colour disappears. A fat-free control sample is tested in parallel. The number of peroxides is expressed in meq/kg.

TBAR

The most commonly used method for measuring oxidative changes in food product is thiobarbituric acid (TBA) test (Shmedes, 1989) based on a spectrophotometric quantitation of a red-violet complex formed with malondialdehyde (MDA). The TBA test measures MDA produced due to the oxidation of fatty acids with three or more double bonds. The concentration of these products may be assessed by red condensation products that absorb at 532–535nm (Spectrophotometer Jenwey 6305 UV).

Hardness

Hardness of salmon was measured using a texture analyser (TA1 Texture Analyser, The Lloyd Instruments/Ametek) in compression mode with a Warner Bratzler shear blade set. The test speed was 100 mm/s.

Measurement of pH

Following the ISO 2917:1999 standards (Measurement of pH), a 10 g sample was pummelled with 90 ml of distilled water for 30 minutes. The pH was measured at 20 ± 1^0C, using HI 2210 pH meter (Hanna Instruments) fitted with a glass electrode.

Statistical Analysis:

The analyses were carried out in triplicates and subjected to one-way ANOVA using Statistica-7. The mean values have been represented at $p<0.05$ level.

RESULTS AND DISCUSSION

Physical and chemical properties determine the technological, culinary and nutritional value of fish. The physicochemical results of the fish analysis are presented in Figure 1 and Figure 2.

The highest moisture content was determined in fresh salmon (68.85%). As seen from the results obtained of the study (see Fig. 1a), when the samples were stored from the first week to the fourth, the moisture content decreased both in salmon salted with NaCl and with KCl. No significant effect of the packaging environment on the moisture content of the product was observed. On average, the moisture content decreased to 13%. The type of salt and its content had no effect on moisture content. Higher CO_2 evolution was observed in samples with higher CO_2 content. This does not reduce the moisture content of the product, but makes the packaging of the product unattractive (Dondero et al., 2004).

The hardness determination results (see Figure 1b) showed that the longer the salmon is kept packaged, the higher the hardness is. This confirms the results of the moisture test. As the moisture content decreases, the hardness of the product increases. The samples with KCl were harder than the samples salted with NaCl. Products packaged in a modified atmosphere were also harder. As the CO_2 content in the in the package decreased, the hardness increased. It can be explained by the influence of CO_2 on the activity of the product and the redistribution of moisture in the packaging.

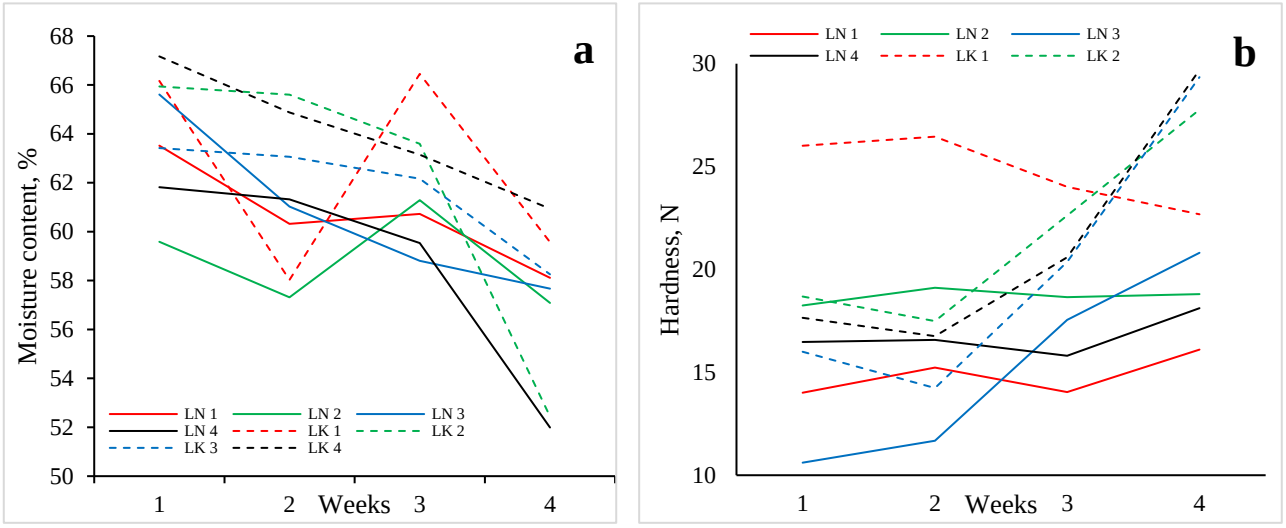

Figure 1. Effect of storage period and packaging type on moisture content (a) and hardness (b) of cold-smoked salmon cured with NaCl and KCl.

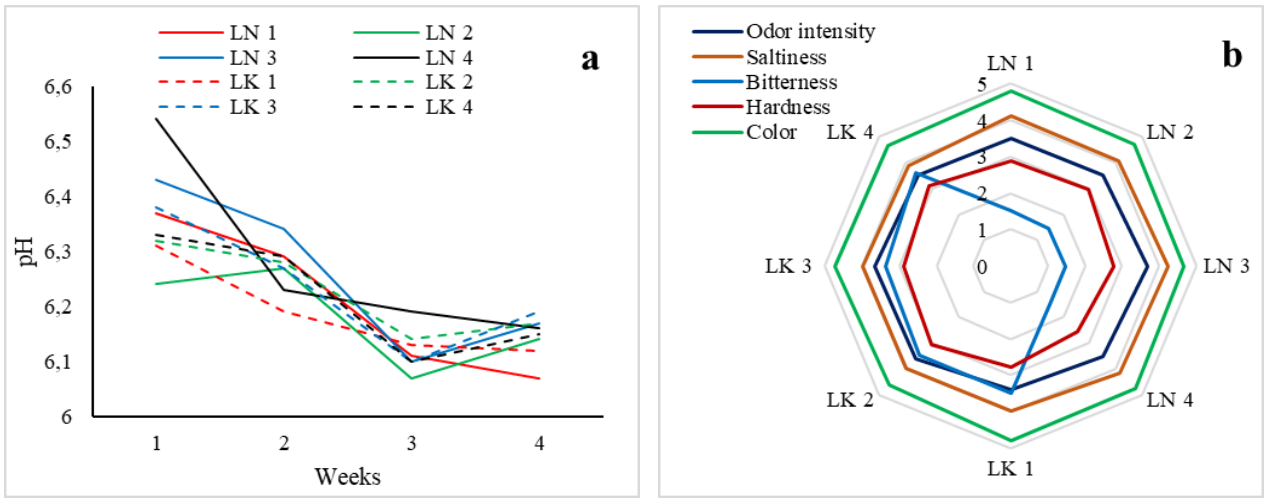

Figure 2. Effect of storage period and packaging type on pH (a) and sensory evaluation (b) of cold smoke salmon cured with NaCl and KCl.

Figure 2a shows the change in acidity of cold-smoked salmon. pH is an important quality indicator showing the possibility of longer storage and some technological properties. Studies have shown that the highest pH was reached with fresh salmon before smoking at 6.49. A decrease in acidity and acidification of the product (lowest pH values of 6.07-6.1) were observed after storing the product for four weeks. This is due to the natural glycolysis processes that produce lactic and other organic acids that alter the pH of the product. The lowest pH at the end of storage is detected in vacuum-packed salmon. The most intense decrease in pH was observed after two weeks of storage. As moisture decreases, the concentration of acids in the products occurs and this alters the pH of the product (Müller, Hans, Huss, Gram, 1998).

While carrying out the organoleptic evaluation the main indicators were observed: odour intensity, saltiness and bitterness, colour and overall hardness of the product. The organoleptic evaluation was carried out a week after smoking. As seen from Figure 2, the products were almost indistinguishable in terms of odour intensity, colour intensity, saltiness and hardness. These characteristics were not very pronounced and the panellists could not capture the difference. According to the bitterness of the product, the products made using KCl were more distinguishable. However, the panellists emphasized that this bitterness is not repulsive

and does not make the product inedible. The panellists did not find any difference between the packaging methods.

The oxidative stability of cold-smoked salmon stored under different conditions was measured by changes in the number of peroxides, fatty acids, and early oxidation products measured by reaction with 2-thiobarbituric acid.

Peroxide formation was observed in all samples when evaluating oxidation changes in terms of peroxide value (see Figure 3). Their accumulation was affected not only by the different method of packaging, but also by the salt used for curing. At the end of storage, after four weeks, samples salted with KCl and vacuum-packed (LN1 and LK1) and with 30% CO_2 + 70% N_2 gas (LN4 and LK4) accumulated three times less peroxide. When storing the products using a gas mixture of 70% Co_2 + 30% N_2, the result difference of the samples with different salts was insignificant. When CO_2 gas was 100% (LN2), twice as much peroxide was formed in the samples with KCl. Comparing the stability of the samples with different salts in terms of peroxide formation, the results show that CO_2 gas reduced the formation of these compounds in the samples with NaCl.

Assessing the effect of gas on oxidative stability, the results indicate that in the samples with NaCl (see Figure 3a), the least peroxide accumulation was achieved when 100% CO_2 gas was used (0.07 meq / 100 g), higher - when CO_2 was reduced to 70% and supplemented with 30% N_2 (0.13 meq / 100 g), mainly with a gas mixture consisting of 30% CO_2 + 70% N_2 (0.23 meq / 100 g).

In salmon samples with KCl, the value of peroxides was highest when packed with CO_2 (0.13 meq / 100 g) and lowest when vacuum (0.05 meq / 100 g). CO_2 gas promoted the formation of peroxides in the samples salted with KCl.

M.Milijašević, J.Babić and other researchers (Milijašević, 2017) conducted a number of the studies of fish packed using different gases and found that peroxides almost do not exist or peroxide number is very low in the first days of storage. However, with longer storage, the more oxygen is in the fish environment, the faster peroxides accumulate. In their studies, packaging of common carp (Cyprinus carpio) at 90% CO_2 + 10% N_2 slowed the proteolytic reaction and secondary lipid oxidation. Based on these indicators, packing carp with 90% CO_2 + 10% N_2 is a more appropriate method than packing 80% O_2 + 20% CO_2 in a gas mixture.

The results of the acid number evaluation (see Fig. 4) show that fat oxidation and hydrolysis were more intense in the samples with KCl. Acid number values after four weeks of storage ranged within 2.2-2.8, and with NaCl - 1.6-2.0 mg / 100 g NaOH. In the samples cured with table salt (see Fig. 4 a), most of the free fatty acids accumulated when packaged under vacuum. In the samples with gases and their mixtures, the number of fats was insignificantly lower, but no effect of different gases was observed.

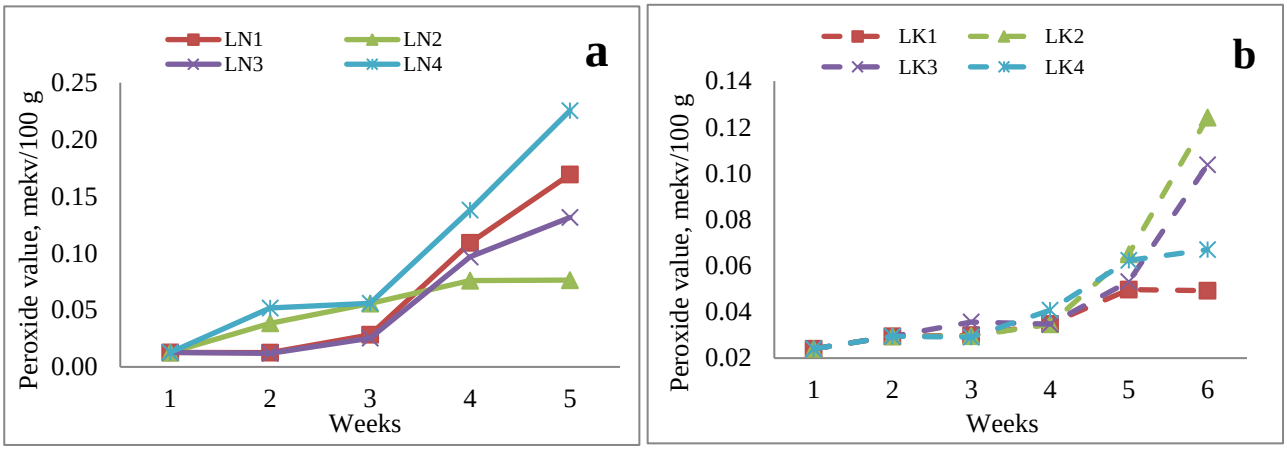

Figure 3. Changes in peroxide number in cold-smoked salmon samples depending on packaging and curing a) with NaCl; b) with KCl

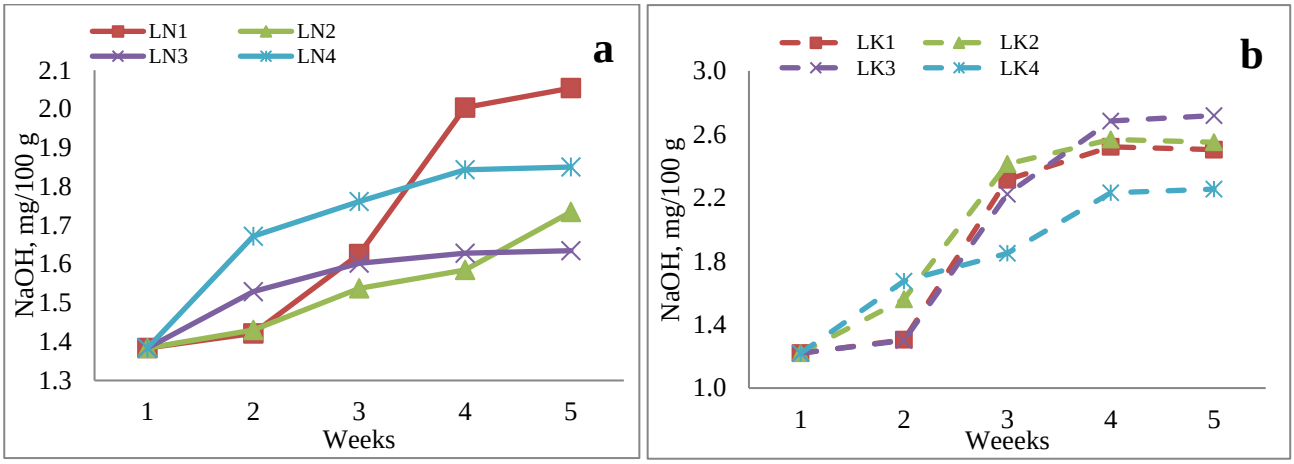

Figure 4. Change in acid number in cold-smoked salmon samples depending on packaging and curing: a) with NaCl; b) with KCl

When cold-smoked salmon with KCl (see Fig. 4 b) was stored, the tendency of acid accumulation was similar in all samples after two weeks, and slightly less acid was formed in three or four weeks in the presence of a gas mixture of 30% CO_2 + 70% N_2. NaCl is a more effective tool of maintaining the oxidation stability of fats in terms of the formation of free fatty acids.

Researchers Rodríguez A., Trigo et al. (Rodríguez et al. 2009) conducted studies on vacuum-packing salmon and identified that the formation of free fatty acids was not affected by packaging, and differences were observed only due to differences in fish composition. This corresponds to the results obtained in our studies that the effect of the gas mixture on the accumulation of free fatty acids is very small. However, according to the results obtained, the vacuum partially inhibited the primary fat oxidation process, e.g. peroxide formation. Comparison of our results shows that the data match, as the number of peroxides in salmon packed in vacuum is the lowest when KCl salt was used for curing (see Figure 3b).

Determination of the degree of oxidation with 2-thiobarbituric acid (TBA) shows the early stages of fat oxidation, in contrast with previous studies. The reaction carried out shows the amount of secondary oxidation products. The obtained results indicated (see Figure 5) that accumulation of oxidation products was twice less in comparison with salmon samples salted with KCl when evaluating after four weeks of storage. The measured absorbance of the compounds formed after the reaction of the oxidation products with TBA at 532 nm wavelength did not show any dependence on the gas or their mixture.

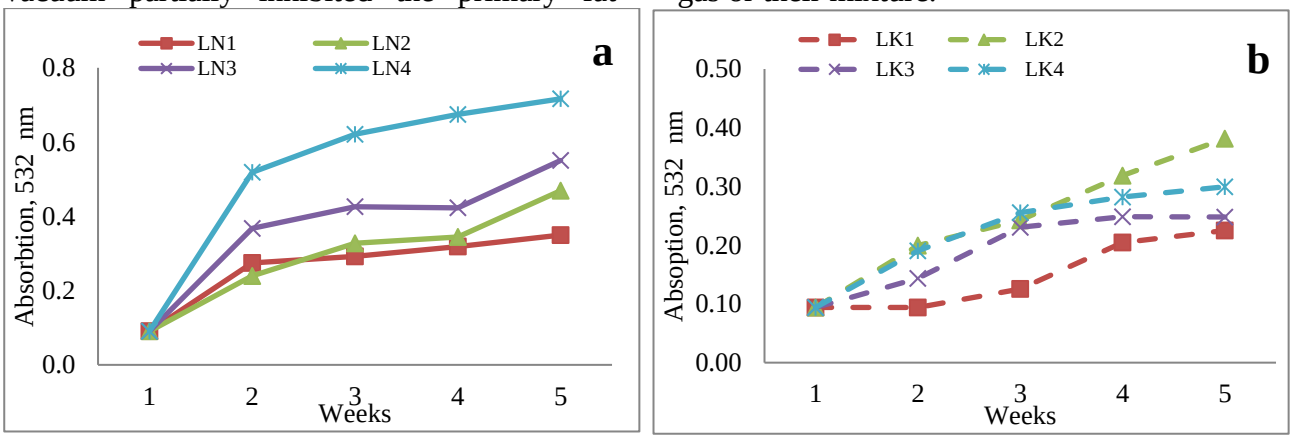

Figure 5.TBA change in cold-smoked salmon samples depending on package and curing: a) with NaCl; b) with KCl

The lowest absorption was in the samples with KCl and vacuum-packed, and the highest with CO_2 gas (see Figure 5 b). The opposite results were found in the samples with NaCl (see Fig. 5 b) - in the samples with 100% CO_2 gas oxidation products accumulated the least (absorption 0.48) and with decreasing the amount of these gases the oxidative stability decreased (absorption increased to 0.72).

Secondary oxidation processes are most effectively inhibited by vacuum, as all samples contained the least amount of secondary oxidation products throughout the period. Vacuum packaging was the most active in terms of oxidation processes according to all assessed indicators during the first weeks of storage (two weeks).

Rodríguez and Trigo (Rodríguez et al. 2009) also analysed the accumulation of secondary oxidation products in vacuum-packed salmon based on the TBA method. The obtained data showed that very little secondary oxidation products were formed, thus conclusions were drawn that the vacuum inhibits the formation of fat secondary oxidation products. Our TBA results also show that the least amount of secondary oxidation products formed in the vacuum-packed samples, regardless of the type of salt used for curing.

Ruiz-Capillas and C, Moral A. (Ruiz-Capillas et al. 2001) analysed the oxidation indices of hake when storing fish in different gas mixtures. Based on their results obtained, mixtures of different gases from CO_2, N_2 and O_2 did not affect the accumulation of secondary oxidation products measured according to TBA.

CONCLUSIONS

Having analysed the quality indicators of cold-smoked salmon fillets and comparing the obtained results, it can be stated that:

1. Shelf-life has an effect on the reduction of the moisture content of the product and, consequently, increase of its hardness. The products were harder using KCl and packing at a lower CO_2 concentration in the mixture. The hardest salmon samples were LK3 and LK4 (29.35 and 29.69 N, respectively) at the CO_2 concentration in the mixture (50% CO_2: 50% N_2 and 30% CO_2: 70% N_2, respectively).
2. After four weeks of storing salmon, the acidity decreased, the pH shifted from 6.49 to 6.07 - 6.19.
3. When carrying out the organoleptic evaluation of the products, so significant differences were determined in terms of the intensity of odour, saltiness and hardness, regardless of the method of packaging. However, products made with KCl were found to be seven times bitterer than products made with NaCl.
4. Based on the results obtained, the oxidation indicators show that the least peroxides are formed in vacuum-packed cold-smoked salmon samples and salted with KCl (0.05 kb / 100 g), and the CO_2 gas present in the package promotes peroxide formation.
5. NaCl is a more effective tool of maintaining the oxidation stability of fats in terms of the formation of free fatty acids. No effect of different gases used for salmon packaging on the formation of free fatty acids was observed.
6. The secondary oxidation processes, as measured by the reaction with TBA acid, are inhibited by vacuum, as these samples contained the least amount of secondary oxidation products in the entire period of storage (absorption 0.23-0.33).

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