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# QUALITY OF SEA BASS MEAT DURING STORAGE ON ICE

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**SUMMARY**

*The goal of this work was to determine the sustainability of the sea bass meat during its storage on ice in the period of twelve days by following sensory, physical- chemical and microbiological parameters of quality and hygienic safety. Thirty sea basses were researched for that goal on the 2<sup>nd</sup>, 4<sup>th</sup>, 6<sup>th</sup>, 9<sup>th</sup> and 12<sup>th</sup> day of storage on ice at 3° C. Sensory grade of quality was evaluated by Quality Index Method test (QIM) created for sea bass, Dicentrarchus labrax (Icelandic Fisheries Laboratories) and Market test. Fish samples were researched on the total count of aerobic mesophyllic bacteria, psychotrophic bacteria and Pseudomonas spp. There were also measured pH- values and the quantity of ammonia. The quantity of ammonia was increasing during the storage of fish and in that manner it was following its sensory characteristics, so the fish samples at the end of our research were evaluated as fish*

*at risk of rotting with 16.1 mg% of ammonia. Together with the increase of aberration from the characteristic appearance and smell of fresh sea bass, the number of psychotrophic bacteria and Pseudomonas spp was increasing concurrently.*

**Key words:** shelf life, sea bass meat, quality

**INTRODUCTION**

Fish has always had an important place in human nutrition and it was a desirable foodstuff not only because of its gastronomic characteristics but also because of the fact that it is food of high nutritional values. First of all, it applies to fish proteins whose value primary lies in easy digestion, better usability and more appropriate amino acid composition, especially when it comes to essential amino acids. Fish fats contain from 60- 84% of unsaturated fatty acids, and beside that, fish is rich in vitamins

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▼ **Table 1.** Autolytic changes in chilled fish (Huss, 1995; Muramoto, 1989; Bremner and Hallett 1985)

Enzymes	Substrate	Changes	Prevention
Glycolytic enzymes	Glycogen	Production of lactic acid, pH of muscle drops, loss of water-holding capacity in muscle High temperature of rigor may result in gapping	Fish should be allowed to pass through rigor at temperatures as close to 0°C as practically possible Pre-rigor stress must be avoided
Autolytic enzymes, involved in nucleotide breakdown	ATP ADP AMP IMP	Loss of fresh fish flavor, gradual production of bitterness with Hx (later stages)	Fish should be allowed to pass through rigor at temperatures as close to 0°C as practically possible Rough handling or crushing accelerates breakdown
Cathepsin	Proteins, Peptides	Softening of tissue-makes processing difficult or impossible	Rough handling during storage and discharge of fish
Chymotrypsin, trypsin, carboxypeptidase	Proteins, Peptides	Autolysis of organs of visceral cavity	Problem increases with freezing/thawing or long-term chill storage
Calpain	Proteins	Softening (crustaceans)	Lack of calcium
Collagenases	Connective tissue	"Gaping" of fillets, softening	Connective tissue decomposition related to time and temperature of storage
TMAO demethylase	TMAO	Formaldehyde-induced toughening of frozen fish	Storage of fish at temperature $\leq 30$ °C, Physical abuse and freezing/thawing accelerate formaldehyde-induced toughening

(A, D, E, B- complex) and mineral substances. Chemical composition of fish significantly varies depending on not only on species of fish, but on nutrition, age, sex, migration, environmental conditions and season as well.

Quality of fish is, first of all, an economic category regulated by the species, categorization of the sensory characteristics i.e. smell and appearance of eyes, gills, skin, mucus, peritoneum and anus, then of the consistency and meat color, and the mode of processing (evisceration, etc.). Among sensory changes which include the smell, appearance, texture and taste of fish meat, the most obvious is the appearance of rigor mortis, and it depends on many factors, such as the temperature of water and storage, handling with the fish, size and physical condition of the fish, and the method of paralyzing or catch (Abe and Okuma, 1991). The texture of fish cooked before the rigor is very soft, it is very stiff but not dry during the rigor, and after the rigor it is firm, juicy and elastic.

After the fish dies, the metabolism goes from aerobic to anaerobic, which significantly reduces the production of

energy. Changes which appear during autolysis are very different, but the most important ones apply to reserves of energy, proteins, lipids and nucleotides (Table 1.).

The process of complex chemical and biochemical processes which appear after the fish dies and affect the characteristics of a fish can be divided to four phases (Huss, 1995):

1<sup>st</sup> phase: fish is fresh and has a sweetish, pleasant taste, sometimes mildly metallic. With some species of fish (cod, flounder) the sweetish taste is the most prominent two- three days after the catch;

2<sup>nd</sup> phase: the loss of the characteristic taste and smell of fish, the meat is of neutral smell and taste, but it still doesn't deviate. The texture is still pleasant;

3<sup>rd</sup> phase: signs of rotting are noticed, followed by the unpleasant smell of substances which appear during decomposition, depending on the fish species and the type of decomposition (aerobic, anaerobic). One of the evaporable compounds can be a trimethylamine (TMA) which appears by bacterial decomposition of trimethylam-

inoxide (TMAO). TMA gives a very characteristic “fish-like” smell. At the beginning of this decomposition phase, the smell and taste can be mildly sour, similar to the smell of cabbage, ammonia, or unpleasant smells of rotten and rancid fish;

4<sup>th</sup> phase: fish can be characterized as rotten.

The postmortem decrease of pH- value affects the physical characteristics of meat since the surface charge of proteins is decreasing, they denature and their capacity of binding water decreases, which has a significantly negative effect on meat texture. So Love (1975) described a connection between the pH- value and firmness and toughness of fish meat (and the loss of water during cooking), where the unacceptable levels of stiffness appear with lower pH- values. Rigor mortis always ends with a “relaxation” of muscles, which is credited to the effect of proteolytic enzymes on actomyosin complexes. Products of autolysis of proteins are peptides of low molecular mass and free amino acid which, except for the fact that they affect the freshness of fish, create favorable environmental conditions for the growth of bacteria of decay (Aksnes and Brekken, 1988). Autolytic changes also appear during the activity of proteolytic enzymes, such as cathepsins, coagulases and calpains.

Although white- flesh fish has relatively little lipids and rotting depends on protein fractions first of all, oxidation and hydrolysis of lipids (bacterial and cell enzymes) are also important because they result in different unpleasant smells and tastes of rancidness, as well as in yellowish discoloration of tissue because of creation of aldehydes, ketones, alkanes, and short chain fatty acids.

## BACTERIAL CONTAMINATION OF FISH

The level of bacterial contamination of fish depends on the environment and bacteriological quality of water where the fish was caught. Many factors affect the microflora of fish, and the most important ones are the temperature of water, quantity of salt in water, nearness of settlement to areas of catching, quantity and origin of food that the fish is fed on, and the method of catch. We find microorganisms on the outer surface (skin, gills) and in the guts of live and just caught fish while the meat was being sustained by a sterile immune system. The entire number of microorganisms varies significantly (102 - 107 cfu/cm<sup>2</sup> of skin, 103 – 109/g of gills and guts). Psychotrophic flora is expected in the Adriatic Sea, where the optimal growth is at 25°C, but it is also capable to grow at 6°C. The most prominent psychotrophics are *Pseudomonas*, *Moraxella*, *Acinetobacter*, *Schewanella* and *Flavobacterium*, and then *Vibrio*, *Photobacterium* and *Aeromonas* (Liston, 1980; Schewan, 1962, 1977). There are two large

groups of bacteria of public- health importance which can contaminate the fish at the time of catch – those which are normally present in water and sea (autochthonous microflora) and those which enter the environment through waste waters, industrial waters, or those from the households. Autochthonous bacteria which can become a risk for health are *Aeromonas hydrophyla*, *Clostridium botulinum*, *Vibrio parahaemolyticus*, *Vibrio cholerae*, *Vibrio vulnificus* and *Listeria monocytogenes* (WHO). The other bacteria of public- health importance include the members of the Enterobacteriaceae family (*Salmonella spp.*, *Shigella spp.*, *Escherichia coli*). Other species of bacteria which can cause human diseases, and they are occasionally isolated from fish are *Edwardsiella tarda*, *Pleisomonas ahigeloidea* and *Yersinia enterocolitica*. Autochthonous pathogen bacteria are present in fresh fish in small number the most often, so if the fish is adequately culinary prepared, and products are properly thermally processed, the risk for food safety is insignificant. During the storage, the usual microflora – bacteria of fish decay, will overgrow pathogen bacteria, therefore the fish will rot before the fish meat decomposes to toxic products, so the consumers will not use it. The risk of those pathogen bacteria can be controlled through applying proper thermal processing which is enough for these bacteria to be destroyed, or storing fish at chilling temperatures with the avoidance of cross- contamination (Huss, 1995).

Microbial pollution is actually mostly only on the surface because it has been found that a small number of bacteria can be found in meat of fish stored on ice (Murray and Schewan, 1979). Ruskol and Bendsen (1992) proved that bacteria in fish meat can be determined if their number on the surface exceeds 10<sup>6</sup> cfu/cm<sup>2</sup>. Psychotrophic bacteria enter the exponential phase of growth immediately after the fish dies and during its storage on ice because they are already adapted to cooler temperatures. Flora changes during the storage, so at aerobic storage on ice after one to two weeks it almost exclusively consists of *Pseudomonas spp* and *S. putrefaciens* (Morita, 1975; Devaraju and Setty, 1985). In order to come to the fish rotting at aerobic storage, 10<sup>8</sup>-10<sup>9</sup> cfu/g of specific bacteria of decay are necessary.

Biochemical researches during the storage and fish rotting show that most of the evaporable compounds are produced by bacteria (Schewan, 1962). The most prominent ones are TMA, sulphur- compounds, aldehydes, ketones, esters and hypoxanthine. Substrates for their production are carbohydrates (lactates and ribose), nucleotides (IMP and inosine), and nonprotein nitrogen compounds, especially amino acids for the production of ammonias and sulfides. The appearance of certain compounds depends

▼ **Table 2.** Quality Index Method test – Sea bass (*Dicentrarchus labrax*) (According to Emilie Martinsdottir, Sc.D., 2002; Icelandic Fisheries Laboratories)

Parameter of quality	Description	Points	
Skin	Appearance /Color	Shiny, iridescent	0
		Dull, beginning of discoloration (head)	1
		Green, yellowish (abdomen)	2
	Smell	Fresh, seaweed, neutral	0
		Metallic, like cucumber	1
		Sour, stale	2
		Rotten, rancid	3
	Texture	<i>Stiff, in rigor</i>	0
		Marks quickly disappear	1
Marks stay longer than 3 seconds		2	
Eyes	Pupil appearance	Clear, black, metallic shine	0
		Grey	1
		Dull, grey, opaque	2
	Shape	Convex	0
		Flat	1
		Concave	2
Gills	Color	Red, orange	0
		Light red, pink, brown	1
		Grey, brown	2
	Mucus	Transparent	0
		Milky, thick	1
		Brown, coagulated	2
	Smell	Fresh, seaweed, neutral	0
		Metallic, grass- like	1
		Sour, musty, stale	2
		Rotting	3
Meat, fillet	Color	Transparent, bluish	0
		Waxy, milky	1
		Nontransparent, yellow, brown spots	2
Viscera	Appearance	Complete viscera	0
		Beginning of decomposition	1
		Decomposed	2
Index of quality		0 - 22	

on specific microorganisms of decay, because they are the only ones which have an activity strong enough to produce the quantity of evaporative compounds which can be noticed organoleptically. The total product of proteolysis or the total volatile basic amines are among the most used methods of evaluation of sea food generally. The method doesn't show the type of decay (bacterial or autolytic), but the growth of proteolytic products is in relation to the number of microorganisms (Kyrana and Loufovois, 2002).

## MATERIAL AND METHODS

For the purpose of this study, 30 sea basses were caught in the Adriatic Sea weighing from 200 to 400 g. Evaluation of quality and hygienic safety of fish was done per six sea basses on the 2<sup>nd</sup>, 4<sup>th</sup>, 6<sup>th</sup>, 9<sup>th</sup> and 12<sup>th</sup> day. It included sensory evaluation (cooking trial, baking trial, QIM and Market- test), physico-chemical (pH- value, determining ammonia quantity) and bacteriological analysis (aerobic mesophyllic bacteria, psychotrophic bacteria, *Pseudomonas spp.*).

Sensory evaluation of quality was performed using the Quality Index Method (QIM) test created for sea bass, *Dicentrarchus labrax*, (Emilie Martinsdottir, Sc.D., at Icelandic Fisheries Laboratories). QIM includes sensor indicators of fish freshness and their assessment using system of points. Fresh fish has the minimum (0), whereas the maximum number of points for the freshness of fish is 22 and it applies to rotten fish. The test estimates appearance of the fish, eyes and gills on the indicators shown in Table 2. At the same time, sensory characteristics of the cooked and baked fish were also evaluated by the Market- test. The evaluators had to decide whether they would buy the fish on the market if it were offered to them, based on the results of cooking and baking trial. Characteristics of bouillon were evaluated by cooking trial and characteristics of fish meat by baking trial according to Živković (1989). Seven evaluators took part in the evaluating group.

The pH- value of meat was determined by the digital pH- meter within physico-chemical analysis, whereas the quantity of ammonia was determined by quantitative microdiffusional procedure by Schmidt (Živković, 1965).

The total count of aerobic mesophyllic bacteria was determined within the bacteriological analysis, which was included by microbiological standards of the existing regulations (Regulation on Microbiological Standards, National Gazette 125/03) (Plate Count Agar - PCA, 30° C, 72 h). Also, the total count of psychotrophic bacteria was determined (PCA agar, 6,5 °C, 10 days), and the number of *Pseudomonas spp.* (*Pseudomonas* agar F, 25 °C, 2 days).

▼ **Table 3.** Number of microorganisms (log<sub>10</sub> cfu/g)

Microorganisms	2. day	4. day	6. day	9. day	12. day
Total count of aerobic mesophyllic bacteria	5.46	5.62	5.59	5.71	6.2
Total count of psychotrophic bacteria	4.29	4.79	5.15	5.46	7.26
<i>Pseudomonas spp.</i>	3.90	4.08	4.48	5.44	5.51

▼ **Table 4.** of physical results- chemical researches (average values)

Physical- chemical parameter	2. day	4. day	6. day	9. day	12. day
pH- value	6.25	6.14	6.13	6.13	6.11
Ammonia (mg %)	9.10	10.4	13.40	15.90	16.10

## RESULTS

After the completion of sensor analysis, all fish samples were evaluated on the 2nd day as the first class fish with insignificant differences (QIM- 0-2 points). Changes of shape of eyes and appearance of the pupil, color and odour of gills and the appearance of gill mucus started to appear already on the 4th day of storage (mean value of QIM- test – 3.5 points). However, during the entire twelve days of storage on ice, the fish showed no prominent sensory changes in the sense of a rotten fish, and on the last day of storage they were scored 10 – 14 points. By the Market- test on the 12th day of storage, 75% of researched fish samples were evaluated as unacceptable for consumption.

The results of bacteriological analysis are shown in Table 3. The total count of aerobic mesophyllic bacteria didn't grow significantly during the ice storage of sea bass and it ranged from 5.46 log<sub>10</sub> CFU/g (2nd day) to 6.2 log<sub>10</sub> CFU/g (12th day). The total count of psychotrophic bacteria increased from 4.29 log<sub>10</sub> CFU/g (2nd day) to 7.26 log<sub>10</sub> CFU/g on the 12th day of storage, when it exceeded the total count of aerobic mesophyllic bacteria. At the same time, an increase in the number of *Pseudomonas spp.* was recorded in the fish meat, which amounted to 5.51 log<sub>10</sub> CFU/g, in comparison to 3.9 log<sub>10</sub> CFU/g on the 2nd day of storage.

There weren't any significant changes of pH- values during the storage of sea bass, so per individual fishes they were from 5.98 to 6.36 as the highest. The quantity of ammonia was increasing during the storage of fish from 9.1 mg% on the 2nd day of storage, to 16.1 mg% on the last day of storage (Table 4.).

## DISCUSSION

During the storage of twelve days at the temperature that didn't exceed 3°C at the evaluation of sensory char-

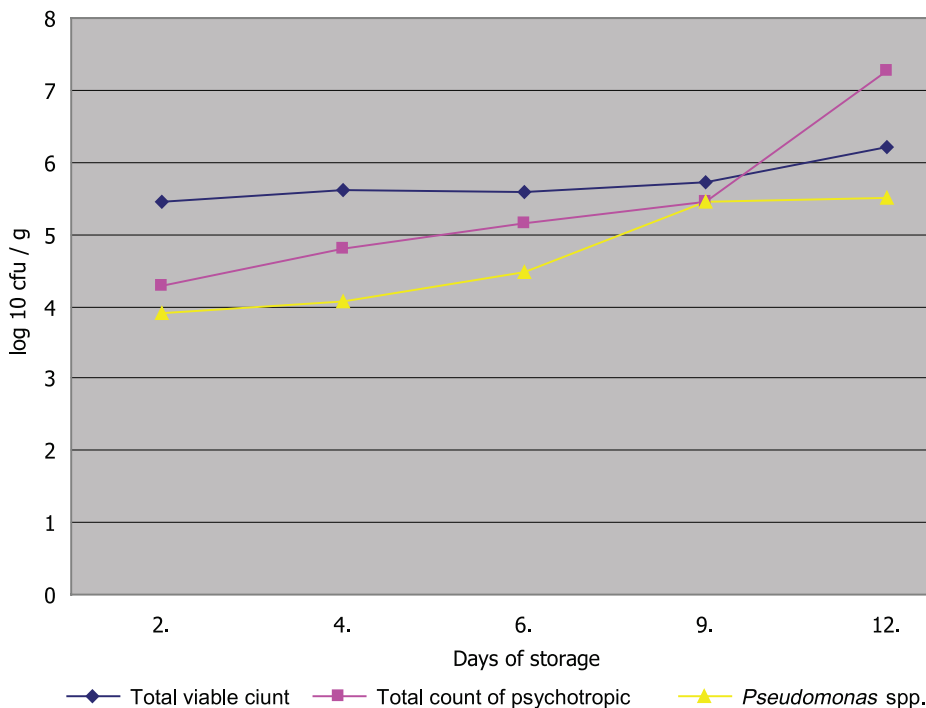
acteristics, the fish samples had satisfactory characteristics although on the fourth day already and especially on the sixth day of storage it showed aberration from the characteristic appearance and the smell of fresh sea bass. So Kyrana and Lougovois (2002) determined that sustainability of noneviscerated sea basses lasts 19 days if stored on ice. Martinsdóttir et al. (2002) in their sea bass sustainability researches based on the sensory grade of cooked fish concluded that sea bass stored at flaky ice can be sustained for 22 days, and sea bass stored on liquid ice for 24 days.

The pH- value in fish samples ranged from 5.98 to the most 6.36, with the tendency of growth after the 6th day of storage. Parisi et al. (2002) determined in their fish sustainability researches that the average pH- value of fish meat is 6.52, i.e. 6.29 in meat of fish killed by hypothermia (stored at 1 and 4 °C). Depending on the storage, the fish was sustainable through 11 days (1 °C), that is, 7 days (4 °C). Kyrana and Lougovois (2002) concluded that the pH- value doesn't change significantly during the first 9 days of storage of sea bass, and its sustainability is estimated on 19 days at the most. In our research the pH- value of different fish meat varied, which could be connected to the fact that fishes didn't weigh equally.

The quantity of ammonia was increasing during the storage of fish, and it was showing the tendency of growth of 9.4 mg% at the beginning to 17 mg% on the last day of storage, with occasional aberrations and falls. Organoleptically faultless fish contains less than 10 mg%, whereas the rotten one contains > 20 mg% NH<sub>3</sub> (Živković, 1989). Therefore, fish samples in our research were evaluated as fresh, long- standing, or the fish at the risk of rotting. The determined quantity of ammonia wasn't completely following sensory characteristics of fish. Namely, on the first day some fishes which were evaluated with good grades by the QIM- test, but with 11.9 mg% of ammonia they were evaluated as long- standing fish. Ammonia



▼ **Figure 1.** . The number of microorganisms in the fish during storage



appears with bacterial deanimation of proteins, peptides and amino acids, as well as with the autolysis of AMP, but it is also considered to be a good indicator of later phases of fish rotting.

Generally, we can say that on the 6<sup>th</sup>, that is on the 9<sup>th</sup> day of storage on the surface and in the fish meat the fast growth of psychotropic bacteria starts. At the same time, there was recorded an increase in the number of bacteria of *Pseudomonas* genus. Poli et al. (2006) determined that the number of *Pseudomonas* spp. is 3.393 log<sub>10</sub> CFU/g on the 1<sup>st</sup> day and 8.894 log<sub>10</sub> CFU/g on the 8<sup>th</sup> day of storage. Papadopoulos et al. (2003) determined that in 15 days of storage the number of bacteria of *Pseudomonas* genus reaches 10<sup>7</sup>/g. Koutsoumanis and Nychas (1999; 2000) proved that the main microorganisms of decay of fish from the Mediterranean waters at aerobic storage at the temperature from 0-15°C are bacteria of *Pseudomonas* spp. genus, and the finding is in relation with the increase of nitrogen.

The total count of aerobic mesophilic bacteria in our research ranged from 1.5x10<sup>5</sup>/g to the most of 2.6x10<sup>6</sup>/g on the 12<sup>th</sup> day of storage. The results can be compared to the results of the research of Caklij et al., (2005) who determined number from 2.78 – 7.03 log<sub>10</sub> CFU/g, whereas Poli et al., (2006) determined the highest total number of mesophilic bacteria of 8.606 log<sub>10</sub> CFU/g in

fillets on the 8<sup>th</sup> day of storage. Papadopoulos et al. (2003) determined higher initial number of mesophilic bacteria from 4.0 log<sub>10</sub> CFU/g, which was growing and on the 15<sup>th</sup> day of storage it was 7.0 log<sub>10</sub> CFU/g. Kyrana and Lougovois (2002) determined the total count of mesophilic bacteria from 10<sup>7</sup>/g on the 16<sup>th</sup> day of storage in the samples of noneviscerated sea bass during its storage on ice.

## CONCLUSION

Aberrations from the characteristic appearance and odour of fresh sea bass are in correlation with the results of

bacteriological research (the increase in number of psychotropic bacteria, *Pseudomonas* spp., aerobic mesophilic bacteria) and physico-chemical analysis (increase in the quantity of ammonia, increase of pH- value). In the sensory research (QIM- test), the fish was evaluated on the last day of storage with 10- 14 points, whereas in the Market- test in the 75% of samples it was unacceptable for consumption. The total count of aerobic mesophilic bacteria reached 6.2 log<sub>10</sub> CFU/g, and the total number of psychotropic bacteria increased to 7.26 log<sub>10</sub> CFU/g on the 12<sup>th</sup> day of storage. The number of bacteria of *Pseudomonas* genus on the 12<sup>th</sup> day was 5.51 log<sub>10</sub> CFU/g in the fish meat, in relation to 3.9 log<sub>10</sub> CFU/g on the 2<sup>nd</sup> day of storage.

There weren't any significant changes of pH- value during the storage of sea bass, so they were from 5.98 to 6.36 at the most. The quantity of ammonia was increasing during the storage of fish from 9.1 mg% to 16.1 mg% on the last day of storage.

## ZUSAMMENFASSUNG FLEISCHQUALITÄT VON SEEBARSCH WÄHREND DER LAGERUNG AUF EIS

Das Ziel dieser Arbeit war, die Erhaltung von Seebarschfleisch während der Lagerung auf Eis im Laufe von 12 Tagen zu bestimmen, u. zw. durch die Beobachtung

der sensorischen, physikal-chemischen und mikrobiologischen Parameter der hygienischen und qualitativen Richtigkeit. Zu diesem Zweck wurden 30 Seebarsche untersucht, am 2., 4., 6., 9. und 12. Tag der Lagerung auf Eis auf 3° C. Die sensorische Qualität ist nach Quality Index Method (QIM) gemacht für Seebarsch, *Dicentrarchus labrax* (Icelandic Fisheries Laboratories) und Market Test bewertet. Die Fischmuster sind auf die gesamte Zahl der aeroben Mesophylbakterien, *Physothrophbakterien* und *Pseudomonas spp.* geprüft. Es wurden auch pH-Werte und Ammoniakmengen gemessen. Die Ammoniakmenge hat sich während der Lagerung vom Fisch vergrößert und so die Veränderung dessen sensorischen Eigenschaften begleitet. Am Ende unserer Untersuchungen wurde der Fisch mit 16,1 mg % Ammoniak als Fisch gefährdet vom Verderb deklariert. Parallel mit der Abweichung vom typischen Aussehen und Geruch vergrößerte sich auch die Zahl der *Psychothrophbakterien* und *Pseudomonas spp.*

**Schlüsselwörter:** Qualität, Seebarschfleisch

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