

## Evaluation of freshness parameters in fish intended for speciality foods

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### Abstract

The aim was to monitor the course of chemical changes (total volatile basic nitrogen, trimethylamine, free fatty acids, peroxide value, malondialdehyde content) in fresh chilled and deep-frozen muscle tissue of 4 commercially important marine fish (*Sparus aurata*, *Dicentrarchus labrax*, *Scomber scombrus*, *Salmo salar*). The results of our own laboratory tests are included.

*Fish, shelf life, chemical changes*

### Introduction

Special foods of the sushi or sashimi type made of raw fish have become a regular item on the menu at catering facilities in the Czech Republic. They are traditionally made of meat of fresh marine fish that may come from aquaculture farms or are caught in the open sea or oceans. Depending on where the fish come from, it may take two or three days before it is available to chefs at catering facilities for the preparation of such gourmet meals. The most important criterion of their suitability is therefore the degree of their freshness. The implied assumption is that fish will remain fresh and safe for human consumption over the entire period of their shelf life. In practice at catering facilities, this means that fish must retain their freshness parameters for a period of three days from the date on which they are delivered to the catering facility. The basic veterinary and hygiene requirement is the maintenance of constant low temperatures (–1 to + 2 °C) during transport and storage of fish, and observation of the principles of good hygiene and manufacturing practice during the preparation and sale of speciality foods. In addition to the sensory criteria of maritime fish freshness set forth in Regulation (EC) 2046/1996, the level and intensity of degradation processes can also be evaluated by means of suitable microbiological parameters or chemical changes to the main components of muscle tissues (Giansante et al. 1998). Protein degradation processes can be assessed through increases in the content of total volatile basic nitrogen and trimethylamine (Anastasio et al. 1999), while lipid freshness can be assessed using the intensity of lipolytic (amounts of free fatty acids) or oxidative changes, whose intensity is usually assessed by measuring the concentration of peroxide or malondialdehyde as a secondary product of lipid oxidation (Shahidi and Hong 1991; Fernández et al. 1997).

The main aim of this study was to evaluate the degree of freshness of meat of several species of maritime fish used for the preparation of Japanese culinary specialities in one catering facility using five selected chemical parameters. A secondary objective was to ascertain the level of changes to these parameters after six-month freezer storage of the fish at –18 °C.

### Materials and Methods

Four species of fresh maritime fish supplied to a catering facility specialising in the preparation and sale of gourmet foods such as sushi and sashimi were used in the experiment. A total of eight Gilthead sea bream

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(*Sparus aurata*, Linnaeus 1758), eight European sea bass (*Dicentrarchus labrax*, Linnaeus 1758), twelve Atlantic mackerel (*Scomber scombrus*, Linnaeus 1758) and eight Atlantic salmon (*Salmo salar*, Linnaeus 1758) were analyzed. The fish were examined in a laboratory on the day they were delivered (Day 1) and on the last day of their sale (Day 3). Fish samples intended for freezing were collected on the delivery day (Day 1), wrapped in a microtene bag and aluminium foil, and stored in a freezer at  $-18^{\circ}\text{C}$ . They were analysed after 6-month storage immediately after thawing. The following chemical parameters of freshness were investigated: TVBN (total volatile basic nitrogen in  $\text{mg } 100 \text{ g}^{-1}$ ), N-TMA (nitrogen-trimethylamine in  $\text{mg } 100 \text{ g}^{-1}$ ), FFA (free fatty acids in % total lipid as oleic acid), PV (peroxide values in  $\text{mekv O}_2 \text{ kg}^{-1}$ ) and MDA (malondialdehyde concentrations in  $\text{mg kg}^{-1}$ ). The methods used conformed to the appropriate CSN ISO standards. Statistical significance was evaluated using the ANOVA unifactorial analysis of variance in Excel 2007.

## Results and Discussion

Fresh fish meat ( $\text{pH} > 5.2$ ;  $a_w > 0.95$ ) is a highly perishable food commodity for which strict observation of all veterinary hygiene rules is required, particularly if raw fish is primarily intended for direct human consumption (Directive (EC) 854/2004). In addition to strict microbiological criteria laid down by Regulation (EC) 2073/2005 (*Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli*, histamine), chemical parameters (Regulation (EC) 2074/2005) and sensory requirements of fish freshness (Regulation (EC) 2046/1996) must also be complied with. For the safety of consumers, fish (as well as cephalopod molluscs), both as raw material and finished products must undergo a freezing treatment in order to kill viable parasites that may pose a risk to the health of the consumer. For parasites other than trematodes (*Trematoda*), the freezing process must consist of lowering the temperature in all parts of the product to at least  $-20^{\circ}\text{C}$  for no less than 24 hours, or to at least  $-35^{\circ}\text{C}$  for no less than 15 hours in order to kill causative agents of parasitoses that might be present and which are transmittable from fish to humans (Regulation (EC) 1276/2011).

Food businesses keep marine fish in refrigerating devices at temperatures not exceeding  $+4^{\circ}\text{C}$ , and use them to prepare speciality foods for a maximum of three days after the fish were delivered to the premises. This is the length of time corresponding to the usual shelf life dates in the Czech Republic for placing fish on the market. In view of this, we did not expect to find any significant differences in the parameters investigated in the fish on the last day of their use. The data in Table 1 nevertheless shows that some differences were ascertained in three instances.

On the last date of shelf life, we found significantly ( $p < 0.05$ ) higher levels of TVBN and N-TMA in salmon muscle compared with Day 1. In this case, the higher TVBN level in the muscle may have been caused by a higher level of initial contamination of salmon muscle with psychrophilic microorganisms (particularly of the genus *Pseudomonas* and *Shewanella putrefaciens*) and their more intensive proteolytic activity. The storage temperature in the refrigerator may have fluctuated at the same time, which may have supported their growth and propagation. From the legislative point of view, the elevated TVBN levels ( $19.16 \pm 1.02 \text{ mg } 100 \text{ g}^{-1}$ ) demonstrated in the study are in order, because salmon muscle is considered fresh up to a TVBN value of  $35 \text{ mg } 100 \text{ g}^{-1}$  (Regulation (EC) 2074/2005). In the second case, we demonstrated a highly significantly ( $p < 0.01$ ) increased intensity of secondary lipid oxidation (MDA concentrations) in a Gilthead sea bream, which may have been due to the specific composition of lipids in this fish species, and also to the fact that the fish were not vacuum-packed during storage. A non-significant increase in primary and secondary lipid oxidation was also recorded in the case of Atlantic mackerel, whose meat is characterized by a fairly high fat content with greater amounts of polyunsaturated fatty acids. The values of other parameters investigated were practically identical, which means that the chemical properties of fish

Table 1. Selected parameters of freshness of the following species of marine fish: Gilthead sea bream (*Sparus aurata*, Linnaeus 1758), European sea bass (*Dicentrarchus labrax*, Linnaeus 1758), Atlantic mackerel (*Scomber scombrus*, Linnaeus 1758) and Atlantic salmon (*Salmo salar*, Linnaeus 1758).

Parameter	Species	Stored at +2 ± 2 °C day of delivery (Day 1)	Last day of sale (Day 3)	Statistical significance	Stored at -18 °C 6 months after the delivery date	Statistical significance
		mean ± s.d.	mean ± s.d.		mean ± s.d.	
TVBN	Gilthead sea bream	22.14 ± 0.89	23.17 ± 0.25	-*	21.13 ± 0.20	-*
	European sea bass	23.10 ± 2.84	22.91 ± 2.01	-*	20.08 ± 0.33	-*
	Atlantic mackerel	21.72 ± 1.05	22.18 ± 1.56	-*	21.45 ± 0.73	-*
	Atlantic salmon	16.60 ± 1.77 <sup>a</sup>	19.16 ± 1.02 <sup>b</sup>	$p < 0.05$	20.85 ± 1.85 <sup>B</sup>	$p < 0.01$
N-TMA	Gilthead sea bream	12.63 ± 0.64	13.64 ± 0.98	-*	12.45 ± 0.26	-*
	European sea bass	12.48 ± 1.68	13.53 ± 2.02	-*	12.18 ± 0.49	-*
	Atlantic mackerel	11.70 ± 0.84	12.88 ± 1.59	-*	12.89 ± 0.90	-*
	Atlantic salmon	9.90 ± 1.41 <sup>a</sup>	12.16 ± 1.33 <sup>b</sup>	$p < 0.05$	11.85 ± 2.02	-*
FFA	Gilthead sea bream	1.11 ± 0.19	1.37 ± 0.16	-*	1.95 ± 0.01	-*
	European sea bass	1.42 ± 0.11 <sup>A</sup>	1.33 ± 0.47	-*	2.21 ± 0.11 <sup>B</sup>	$p < 0.05$
	Atlantic mackerel	0.88 ± 0.34 <sup>A</sup>	1.48 ± 0.25	-*	2.93 ± 0.49 <sup>B</sup>	$p < 0.05$
	Atlantic salmon	0.40 ± 0.07 <sup>A</sup>	0.53 ± 0.10	-*	2.73 ± 0.62 <sup>B</sup>	$p < 0.01$
PV	Gilthead sea bream	23.99 ± 0.97 <sup>B</sup>	23.63 ± 0.77	-*	14.61 ± 0.30 <sup>A</sup>	$p < 0.05$
	European sea bass	22.76 ± 0.18	21.05 ± 1.79	-*	6.68 ± 0.75	-*
	Atlantic mackerel	9.60 ± 1.70 <sup>A</sup>	17.65 ± 9.15	-*	40.81 ± 0.06 <sup>B</sup>	$p < 0.01$
	Atlantic salmon	2.25 ± 0.35 <sup>A</sup>	3.14 ± 1.36	-*	61.52 ± 6.30 <sup>B</sup>	$p < 0.01$
MDA	Gilthead sea bream	32.16 ± 0.86 <sup>a</sup> <sup>A</sup>	55.70 ± 8.93 <sup>b</sup>	$p < 0.01$	38.29 ± 6.70 <sup>B</sup>	$p < 0.01$
	European sea bass	5.04 ± 0.84 <sup>a</sup>	6.67 ± 3.79	-*	15.54 ± 4.46 <sup>B</sup>	$p < 0.01$
	Atlantic mackerel	88.23 ± 20.97 <sup>a</sup>	148.98 ± 28.39	-*	201.19 ± 21.29 <sup>B</sup>	$p < 0.01$
	Atlantic salmon	13.36 ± 10.64 <sup>a</sup>	10.35 ± 3.54	-*	41.58 ± 10.68 <sup>B</sup>	$p < 0.01$

TVBN: Total volatile basic nitrogen (in mg 100·g<sup>-1</sup>)

N-TMA: Nitrogen-trimethylamine (in mg 100·g<sup>-1</sup>)

FFA: Free fatty acids (in % total lipid as oleic acid)

PV: Peroxide values (in mekv O<sub>2</sub>·kg<sup>-1</sup>)

MDA: Malondialdehyde concentrations (in mg·kg<sup>-1</sup>)

Parameter values furnished with various indexes <sup>a</sup> (A), <sup>b</sup> (B) are statistically significant at a given level of significance ( $\alpha = 0.05$ ;  $\alpha = 0.01$ ); index <sup>a</sup> (A) always identifies the lowest value of the particular parameter, index <sup>b</sup> (B) identifies the next higher one. Lowercase letters <sup>a</sup>, <sup>b</sup> indicate statistically significant differences between the delivery day and the last day on which the fish were used to prepare speciality foods. Uppercase letters <sup>A</sup>, <sup>B</sup> indicate differences in parameter values between the delivery day and after six-month storage in a freezer at -18 °C.

-\* No statistically significant differences ( $p > 0.05$ ) in the values of the parameters shown were found.

muscle on the third day of sale were similar to those on Day 1 when delivered to the facility.

The results reported for fish stored for 6 months in a freezer at a temperature of -18 °C are typical for this type of storage (Simeonidou et al. 1997; Aubourg 1999; Fagan et al. 2003). While the activity of proteolytic enzymes was reduced in the fish species investigated with the exception of salmon, ongoing lipolytic and oxidative

processes in fish fat were the cause of the differences demonstrated between the pertinent parameters found on Day 1 and after six-month storage.

As is evident from the differences in these values, the intensity of these ongoing processes was different in different fish species, with secondary oxidative processes in fats, however, being predominant, especially in salmon and European sea bass, whose malondialdehyde concentrations after 6-month storage increased more than threefold. In the mackerel, although the value of this parameter in its fat was the highest in absolute terms ( $201.19 \pm 21.29 \text{ mg}\cdot\text{kg}^{-1}$ ), there was only a twofold increase in these values.

## Conclusions

The study showed that for the preparation of speciality foods such as sushi and sashimi, the catering facility used raw materials that, from the point of view of the chemical parameters investigated, can be considered fresh on the last day of their shelf life, i.e. on Day 3 after delivery. As for the dynamics of changes in lipolytic and oxidative processes in fats, however, we can only note their existence but cannot suggest any measures or recommendations for the safety and hygiene evaluation of fish meat usability, because there are no binding limits set forth in the regulations that the relevant chemical parameters (FFA, PV, MDA) are not permitted to exceed.

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