Formation of biogenic amines in fillets and minced flesh of three freshwater fish species stored at 3 °C and 15 °C

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Abstract

The aim of the present study was to compare the dynamics of biogenic amines formation in three freshwater fish species, to compare the progress of chemical changes in filleted and minced flesh, to find correlations of such changes with sensory properties and to find possible quality indicators for selected fish species. Samples (n = 156) of fish fillets and fish mince of common carp (*Cyprinus carpio*), rainbow trout (*Oncorhynchus mykiss*) and perch (*Perca fluviatilis*) were stored at 3 °C and 15 °C. The amines were determined as the *N*-substituted benzamides, after derivatization with benzoylchloride by micellar electrokinetic capillary chromatography. Based on the biogenic amine contents and sensory properties, the shelf life of samples at 3 °C was 11–16 days and 7–10 days for fillets and mince, respectively, and 2–3 days at 15 °C regardless of sample processing. Content of putrescine seems to be a good quality indicator for all examined fish species. The fish species and the method of flesh processing did not have a significant influence on the putrescine formation. Tyramine was formed mainly in carp and trout mince at 15 °C. Samples of good and acceptable quality did not contain toxicologically significant concentrations of histamine or tyramine. Contrary to marine fish, information about biogenic amines contents in freshwater fish is scarce. Comparison of the dynamics of amines formation in fillets and processed fish flesh has not been studied yet. The progress of decomposition processes is compared based on dynamic models.

Putrescine, carp, trout, perch, quality indicator

Biogenic amines (BAs), namely putrescine (PUT), cadaverine (CAD), spermidine (SPD), spermine (SPM), histamine (HIM), tyramine (TYM) and tryptamine (TRM) are organic bases that occur not only in fish and fish products, but also in various other foods of proteinaceous origin. Accumulation of BAs in fish flesh is associated with continuing spoilage (Baixas-Nogueras et al. 2002). The reasons for amine determination in fish are twofold. The first is their potential toxicity; the second is the possibility of using them as food quality indicators (Křížek et al. 2004). Risk of amines formation is high especially when the flesh is minced (Chen et al. 2008). Although numerous studies on the formation of amines in marine fish have been reported, only limited data are available on changes of BAs during spoilage of freshwater fish. For rainbow trout (*Oncorhynchus mykiss*), PUT, TYM, SPD and SPM were recommended as quality indicators (Chytiri et al. 2004). The contents of BAs correlated with the sensory rejection time for rainbow trout (Katikou et al. 2006) and for carp (Křížek et al. 2002).

Common carp (*Cyprinus carpio*), rainbow trout and perch (*Perca fluviatilis*) are important freshwater fish rich in unsaturated fatty acids (Buchtová et al. 2007). In Europe all these species are very popular with anglers and their farm production is large. Fish mince is a prospective raw material for food industry. The aim of the present study was to compare the dynamics of BAs formation in three common freshwater fish species, to compare changes in BAs in filleted and minced flesh, to correlate such changes with sensory properties and to find possible quality indicators for selected fish species.

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Materials and Methods

Fish production and processing

Fresh common carp (*Cyprinus carpio*) (n = 7; average weight: 1.5–2.5 kg; length: 35–50 cm; omnivorous, planktonofagous fish), rainbow trout (*Oncorhynchus mykiss*) (n = 25; 0.25–0.35 kg; 25–30 cm; carnivorous, predatory fish) and perch (*Perca fluviatilis*) (n = 25; 0.30–0.45 kg; 25–30 cm; carnivorous, predatory fish) were obtained from a fish farm in Vodňany near České Budějovice (Czech Republic). The fish were killed, gutted and their body was cut into two halves (a pair of fillets with backbones). The material was washed in drinking water. One half was used for fillets, the second half was processed in a fish meat drum separator to produce fish mince. Fillets were prepared from portions of about 100 g of muscles from the chest area of the fish. Fish mince was produced in the TR-6 meat separator (Nurle Oy, Helsinki, Finland). Fish samples (n = 156) were analyzed in duplicate. Samples were placed in HDPE (polyethylene) bags. Experiments were conducted at 3 and 15 °C. Fish flesh and fish mince samples (n = 156) were analysed in parallel every 24 h of storage.

Analyses and sensory tests

Samples were homogenised in a commercial food hand-blender (Philips, Vienna, Austria). Biogenic amines were extracted from 40 g of homogenised flesh samples with diluted perchloric acid, p.a. (0.6 M). After filtration, the volume was made up to 150 ml with perchloric acid. The amines were determined as the *N*-substituted benzamides, after derivatization with benzoylchloride by micellar electrokinetic capillary chromatography, using capillary zone electrophoresis machine. The procedure has been described in detail by Křížek and Pelikánová (1998). Analyses were carried out using a Spectraphoresis 2000, a fully automated system for capillary zone electrophoresis equipped with a multi-wavelength UV-VIS scanning detector (Thermo Separation Products, Fremont, CA, USA). Sensory tests, carried out by five trained panelists, were complementary to the main objective of the study, the determination of chemical changes in the flesh matrix and were simplified to three levels: good (1), acceptable (2) and poor (3). The organoleptic properties were based mainly on odour and general appearance. Levels of odour: 1 (meaty), 2 (slightly spicy), 3 (fishy, repulsive). Levels of general appearance (colour/texture): 1 (white/tightly elastic), 2 (greyish/solid), 3 (grey/muddy).

Statistical evaluation

Samples were prepared in parallel and each sample was analysed twice. Fitting of curves, describing the kinetics of amine formation, was done by the mathematical software Maple V v. 5.0 (Waterloo Maple Inc., Waterloo, Canada). Correlation coefficients were calculated as Spearman-Rank correlation type (Multivariate analysis, correlation matrix). Analysis of variance (ANOVA) was calculated by General linear models, Up to 1-way model type. These and other statistics were calculated using NCSS 2007 (NCSS, LLC., Kaysville, UT, USA).

Results and Discussion

Mean contents of all amine concentrations, for all sampling days and sensory scores, are shown in Tables 1–4. Relative standard deviations of the results were ± 7–10%. Due to the the large extent of tables, individual values of RSDs are not shown. Experiments were designed as dynamic. Contents of PUT, CAD, HIM and TYM increased with time, SPD showed very slight decrease in carp and perch samples. Sensory properties were highly correlated especially with PUT and CAD in all samples (Table 5). A mutual relationship between sensory score and HIM and TYM was not significant, contrary to our experiments with vacuum-packed carp flesh (Křížek et al. 2004). These and previous results (Křížek et al. 2002) reveal the applicability of PUT or CAD as the quality criterion not only for carp flesh, but also for trout and perch flesh. Cadaverine and HIM are sometimes formed belatedly, compared to sensory signals, which was also observed by Chytiri et al. (2004) in trout samples. Different fish species might reach different BA levels at the sensory rejection time. Information is still lacking. Ozogul et al. (2006) found, that samples of European eel (*Anguilla anguilla*) at the sensory rejection level contained 46–88 mg PUT/kg. For red mullet (*Mullus barbatus*) PUT content exceeding 10 mg/kg was associated with poor quality samples (Ozyurt et al. 2009). Similar value of 13–14 mg/kg was found by Chytiri et al. (2004) studying rainbow trout. Supposing that 15 mg PUT/kg represents a critical concentration (Křížek et al. 2002), we tried to find the respective critical times (days), when the given concentration had been reached.

As the kinetic curves of PUT formation usually show a smooth increase, they can be described by regression equations (Křížek et al. 2004; Baixas-Nogueras et al. 2005). By solving these equations for the proposed critical concentration

Table 1. Mean contents of biogenic amines (mg/kg) in fish fillets kept at 3 °C

	Time (days)																	
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Carp (*Cyprinus carpio*)																		
Putrescine	2.3	2.1	2.4	2.5	1.6	2.1	2.8	4.9	3.4	0.7	3.9	5.6	8.7	10.7	12.7	27.4	33.4	74.7
Cadaverine	ND	ND	ND	ND	ND	ND	ND	ND	7.8	1.0	4.3	3.5	4.2	3.8	4.6	9.6	14.4	26.3
Spermidine	16.1	18.4	14.1	18.2	15.2	15.5	17.7	8.2	10.1	5.2	9.7	7.7	4.1	2.8	5.2	3.5	4.0	4.0
Spermine	2.5	4.2	4.2	2.8	2.5	2.1	1.4	4.1	5.6	1.1	2.8	2.2	2.5	2.3	4.6	4.2	3.5	3.2
Histamine	ND	ND	0.1	ND	ND	0.33	ND	ND	0.55	0.33	ND	ND	0.11	0.44	ND	ND	ND	0.11
Tyramine	1.2	0.56	0.31	1.6	0.94	0.60	0.67	0.59	0.95	1.2	1.3	1.1	0.93	0.90	0.42	0.10	0.32	0.46
Tryptamine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sensory	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	3	3	3
Rainbow trout (*Oncorhynchus mykiss*)																		
Putrescine	3.1	3.3	2.5	3.4	3.1	3.5	3.4	3.8	3.8	3.7	3.1	3.8	4.3	4.9	12.1	16.7	18.7	29.5
Cadaverine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.1	5.3	7.1	27.1	26.6	30.9	38.8
Spermidine	8.1	8.8	7.8	7.7	8.3	10.5	5.6	9.7	9.0	6.5	6.8	7.1	6.1	9.4	6.4	8.2	9.6	6.0
Spermine	7.2	7.7	6.1	6.8	6.5	6.9	4.6	7.2	4.8	5.8	6.3	6.2	7.2	9.0	9.4	4.5	5.2	4.4
Histamine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.03	0.02	0.02	ND	0.27	0.32	0.1
Tyramine	0.21	0.51	0.40	0.37	0.41	0.46	0.79	0.95	0.70	0.52	0.37	0.32	0.34	0.33	6.24	0.61	0.71	2.36
Tryptamine	0.12	ND	0.62	ND	0.22	0.27	ND	0.17	ND	0.10	0.21	0.12	0.07	0.18	0.4	0.08	0.08	0.21
Sensory	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	3
Perch (*Perca fluviatilis*)																		
Putrescine	1.5	2.3	1.4	2.1	1.1	1.7	1.9	2.5	5.4	6.7	13.8	19.8	25.6	31.2	47.5	54.7	74.9	117
Cadaverine	0.60	1.9	ND	0.50	0.62	0.32	0.51	0.72	2.0	3.4	3.4	3.6	3.5	3.8	4.2	8.5	11.8	19.3
Spermidine	3.8	5.1	3.6	3.7	2.7	6.2	3.3	3.4	4.5	1.3	1.2	1.1	1.2	1.5	1.9	3.2	2.8	2.0
Spermine	4.8	6.0	6.0	6.3	6.7	6.8	6.8	8.5	7.7	7.8	6.1	5.1	5.3	4.9	4.8	3.2	2.4	2.9
Histamine	ND	ND	ND	ND	0.13	0.08	ND	ND	ND	ND	0.04	0.05	0.12	0.18	0.17	0.48	ND	ND
Tyramine	ND	0.52	ND	0.46	0.22	0.27	0.57	0.97	1.0	0.37	0.39	0.28	0.51	0.60	0.18	0.19	0.59	0.20
Tryptamine	ND	0.42	ND	ND	ND	ND	ND	ND	0.32	ND	0.21	ND	0.05	ND	ND	ND	ND	ND
Sensory	1	1	1	1	1	1	1	1	1	1	2	2	3	3	3	3	3	3

ND - not detected

Table 2. Mean contents of biogenic amines (mg/kg) in fish mince kept at 3 °C

	Time (days)																	
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Carp *(Cyprinus carpio)*																		
Putrescine	6.4	7.2	6.6	4.3	5.3	6.1	2.7	7.3	6.7	21.6	23.7	34.9	42.6	49.7	83.6	97.9	116	168
Cadaverine	ND	ND	ND	ND	ND	ND	ND	1.4	5.1	5.1	5.7	6.1	8.1	12.7	16.0	18.5	19.5	21.2
Spermidine	9.1	24.2	20.4	14.7	19.3	32.8	19.7	30.3	16.3	17.2	21.5	16.8	26.3	15.8	21.2	27.6	21.6	19.9
Spermine	1.4	4.1	4.0	2.3	4.0	7.6	3.6	6.6	6.4	5.9	6.1	4.2	5.2	6.9	7.0	7.1	5.8	6.1
Histamine	ND	ND	0.42	ND	0.09	ND	ND	ND	ND	0.24	0.43	ND	ND	0.12	0.14	ND	ND	0.23
Tyramine	1.4	0.76	1.4	0.54	0.42	1.2	0.34	7.5	0.51	0.75	1.1	1.1	0.98	1.6	1.2	2.2	0.97	1.2
Tryptamine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sensory	1	1	1	1	1	1	1	1	2	1	2	2	2	3	3	3	3	3
Rainbow trout *(Oncorhynchus mykiss)*																		
Putrescine	13.0	14.0	6.3	14.0	15.6	17.1	13.2	12.5	17.7	21.2	22.4	23.4	25.1	26.7	31.5	39.8	43.2	48.9
Cadaverine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	5.8	15.3	24.9	29.1	36.1	40.1	46.2	54.2
Spermidine	17.7	19.7	22.3	21.9	20.4	21.3	16.8	15.9	23.0	15.4	19.2	17.2	14.9	19.3	24.6	17.5	23.1	17.6
Spermine	5.5	7.2	4.9	7.3	6.3	7.4	4.6	6.1	6.0	5.1	5.1	6.8	5.2	7.1	6.5	4.1	6.0	4.7
Histamine	ND	ND	ND	ND	0.02	ND	ND	ND	ND	ND	0.12	0.12	0.14	ND	0.12	0.14	ND	ND
Tyramine	0.05	ND	ND	0.35	ND	0.02	0.35	0.21	0.48	0.47	0.74	0.56	0.43	1.1	0.78	0.64	1.2	1.2
Tryptamine	ND	ND	ND	ND	ND	0.11	0.12	0.15	0.12	0.11	0.14	0.11	0.13	0.15	0.13	0.11	0.13	0.14
Sensory	1	2	2	3	2	2	3	2	2	3	3	3	3	3	3	3	3	3
Perch *(Perca fluviatilis)*																		
Putrescine	6.4	6.0	6.0	5.9	6.0	6.1	6.3	8.3	9.9	14.8	16.2	18.7	21.6	25.7	53.1	78.9	92.1	146
Cadaverine	ND	0.21	ND	0.32	ND	ND	0.34	0.62	2.6	3.0	10.9	15.8	20.1	25.7	51.4	82.3	127	150
Spermidine	9.7	7.3	8.0	9.4	8.7	8.5	8.0	8.2	5.1	2.4	1.9	1.9	0.95	0.35	1.11	0.89	0.17	0.78
Spermine	5.2	5.2	5.0	5.3	4.3	4.9	4.3	6.1	3.5	4.3	5.7	4.0	5.0	4.1	3.8	2.9	4.2	3.8
Histamine	ND	ND	ND	ND	ND	0.14	0.11	ND	ND	ND	ND	ND	0.05	ND	0.04	0.16	ND	0.15
Tyramine	0.21	0.31	0.33	0.24	0.65	0.75	0.64	0.66	0.23	0.59	0.62	0.54	0.52	0.39	0.43	0.25	0.67	0.72
Tryptamine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sensory	1	1	1	1	1	1	1	1	1	2	2	2	2	3	3	3	3	3

ND - not detected

Table 3. Mean contents of biogenic amines (mg/kg) in fish fillets kept at 15 °C

	Time (days)							
	0	1	2	3	4	5	6	7
Carp (*Cyprinus carpio*)								
Putrescine	2.3	2.0	5.8	37.3	61.0	117	213	236
Cadaverine	ND	0.71	4.4	33.0	55.3	152	310	473
Spermidine	19.4	22.8	16.6	6.9	6.8	4.6	5.1	2.9
Spermine	3.0	3.3	2.6	4.9	4.6	3.4	4.5	4.4
Histamine	ND	0.26	0.18	2.1	26.0	30.9	35.5	38.6
Tyramine	0.05	0.41	ND	3.4	15.5	40.9	74.1	74.7
Tryptamine	ND	0.48	ND	ND	0.91	1.7	4.6	7.5
Sensory	1	1	1	3	3	3	3	3
Rainbow trout (*Oncorhynchus mykiss*)								
Putrescine	3.1	3.4	4.3	19.0	70.0	126	212	330
Cadaverine	ND	0.11	22.4	72.9	84.5	99.9	101	129
Spermidine	8.2	8.1	10.0	7.4	1.7	2.6	1.5	1.5
Spermine	6.0	6.2	8.4	8.7	7.6	5.0	4.9	4.6
Histamine	ND	ND	ND	9.9	50.7	62.8	64.4	79.7
Tyramine	ND	ND	ND	7.8	8.2	38.0	43.9	99.6
Tryptamine	ND	0.13	0.35	0.58	ND	ND	ND	ND
Sensory	1	1	1	2	3	3	3	3
Perch (*Perca fluviatilis*)								
Putrescine	6.4	7.6	33.7	46.7	52.5	71.2	99.6	130
Cadaverine	0.61	1.2	15.9	19.2	22.1	22.5	25.6	33.4
Spermidine	3.9	4.6	2.5	1.1	1.1	0.79	0.44	0.13
Spermine	7.4	7.3	4.2	4.3	4.3	5.5	3.5	3.9
Histamine	ND	0.14	0.22	4.64	46.0	53.8	63.3	110
Tyramine	ND	ND	0.15	0.10	2.3	33.6	58.6	79.0
Tryptamine	ND	ND	1.3	ND	ND	ND	ND	ND
Sensory	1	1	2	3	3	3	3	3

ND - not detected

(15 mg/kg), we obtained estimates of the respective critical times (days). Table 6 shows calculated critical days of storage (CCD) of fish flesh when a given PUT level (15 mg/kg) can be expected. CCD are estimated by solving the equation PUT = (A + B*Time)/(1 + C*Time) which fits best the dynamics of PUT formation (for regression correlation coefficients see Table 6). It is seen that the critical content of PUT was reached in 11–15 days in filleted flesh kept at 3 °C and in 6–10 days in fish mince. At 15 °C the differences between fillets and mince were not so pronounced, but higher temperature shortened this time to 2–3 days. Typical sensory score for given PUT level (15 mg/kg) was 1–2. At 15 °C the difference in the CCD (fillets, mince) was not so marked, compared to samples kept at 3 °C, probably due to the dominant deleterious effect of the temperature.

These results raised the question whether the differences among the CCD are significant. Three factors for the ANOVA tests were selected: fish species (A), the manner of sample processing (B) and the temperature (C). The results were as follows. Factor A: F = 0.13, $P = 0.884$ ($P \gg 0.05$); Factor B: F = 5.13, $P = 0.058$ ($P > 0.05$) and Factor C: F = 38.40, $P = 0.00045$ ($P \ll 0.001$).

It can be summarized that the content of PUT seems to be a good quality indicator not only for carp (Křížek et al. 2002), but also for trout and perch. For this purpose

Table 4. Mean contents of biogenic amines (mg/kg) in fish mince kept at 15 °C

	Time (days)							
	0	1	2	3	4	5	6	7
Carp *(Cyprinus carpio)*								
Putrescine	6.4	5.6	43.1	62.1	108	175	186	228
Cadaverine	ND	0.53	86.7	89.4	114	139	172	242
Spermidine	9.0	16.5	17.6	12.0	11.0	9.0	1.8	2.2
Spermine	3.2	3.3	4.6	4.2	4.8	6.1	2.7	2.8
Histamine	ND	ND	0.23	0.91	2.6	15.3	22.8	33.1
Tyramine	0.05	ND	5.46	3.3	14.6	54.7	74.9	78.7
Tryptamine	ND	ND	2.0	ND	ND	0.97	ND	ND
Sensory	1	3	3	3	3	3	3	3
Rainbow trout *(Oncorhynchus mykiss)*								
Putrescine	13.0	15.9	20.1	56.1	92.3	144	261	398
Cadaverine	ND	ND	ND	36.1	144	280	485	839
Spermidine	8.1	21.9	18.5	12.0	8.6	8.5	4.3	3.7
Spermine	5.5	7.1	3.5	4.7	6.2	5.0	3.9	5.8
Histamine	ND	ND	ND	0.41	6.6	22.5	31.5	34.1
Tyramine	ND	0.09	0.25	7.3	7.3	33.9	51.8	86.5
Tryptamine	ND	ND	ND	0.38	0.38	1.0	6.2	12.2
Sensory	2	2	2	3	3	3	3	3
Perch *(Perca fluviatilis)*								
Putrescine	1.5	2.1	26.5	84.8	203	253	342	419
Cadaverine	ND	1.8	27.0	98.6	229	316	457	552
Spermidine	8.7	5.7	5.3	1.6	2.1	2.6	2.6	2.5
Spermine	5.2	5.4	4.4	3.5	4.2	3.2	2.9	2.6
Histamine	ND	ND	0.26	1.9	10.0	34.6	35.8	36.1
Tyramine	ND	ND	0.81	0.55	0.75	0.17	ND	ND
Tryptamine	ND	ND	0.65	ND	ND	ND	0.41	ND
Sensory	1	1	3	3	3	3	3	3

ND - not detected

CAD can be alternatively used, because both these amines were in the best correlation with sensory levels. Histamine and TYM were formed preferentially in samples at 15 °C and thus their occurrence may signal improper temperature conditions. The CAD, HIM and TYM sometimes showed a slight delay in their formation compared to sensory signals. According to the calculated critical days for the PUT content it was found that all the three fish species do not significantly differ in their susceptibility to spoilage. The mincing accelerated the spoilage especially in samples stored at 15 °C. Fish fillets and fish mince stored at 15 °C showed signs of decay in chemical and in organoleptic indicators in 2 days, fish mince stored at 3 °C in about 8 days, fish fillets stored at 3 °C in about 14 days. Samples of fish flesh and fish mince did not contain toxicologically significant concentrations of HIM or TYM, provided the sensory score of the samples was less than 3.

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Table 5. Spearman correlation coefficients among amines and fish quality sensory score (samples kept at 3 °C)

	PUT	CAD	HIM	TYM	SPD	SPM
Carp/ Fillets	0.835***	0.764***	NS	NS	-0.813***	NS
Carp/ Mince	0.876***	0.932***	NS	NS	NS	0.535*
Trout/ Fillets	0.546*	0.621**	0.603**	NS	NS	NS
Trout/ Mince	0.698**	0.665**	NS	0.745***	NS	NS
Perch/ Fillets	0.890***	0.882***	0.505*	NS	-0.626**	-0.779***
Perch/ Mince	0.919***	0.924***	NS	NS	-0.910***	-0.528*

NS – not significant, PUT – putrescine, CAD – cadaverine, HIM – histamine, TYM – tyramine, SPD – spermidine, SPM – spermine
* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

Table 6. Calculated critical days of storage of fish flesh when a given putrescine level (15 mg/kg) can be expected

	Calculated critical days	r	Sensory score
Carp/ Fillets/ 3 °C	15.0	0.993	2
Trout/ Fillets/ 3 °C	16.4	0.974	1
Perch/ Fillets/ 3 °C	11.0	0.995	2
Carp/ Mince/ 3 °C	8.2	0.990	1
Trout/ Mince/ 3 °C	6.4	0.978	2
Perch/ Mince/ 3 °C	9.8	0.989	2
Carp/ Fillets/ 15 °C	2.7	0.978	2
Trout/ Fillets/ 15 °C	2.9	0.994	1
Perch/ Fillets/ 15 °C	2.0	0.993	1
Carp/ Mince/ 15 °C	1.9	0.986	3
Trout/ Mince/ 15 °C	2.1	0.996	2
Perch/ Mince/ 15 °C	2.1	0.989	2

r – regression correlation coefficient, Sensory score – means sensory score observed
at calculated time (1 – good, 2 – acceptable, 3 – poor)

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