The dynamics of biogenic amine formation in muscle tissue of carp (*Cyprinus carpio*)

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ABSTRACT: The effect of various storage temperatures (3 ± 2°C; 24 ± 1°C and –18 ± 1°C) on the formation of biogenic amines in the muscle tissue of four hybrid lines of carp (*Cyprinus carpio*) with respect to microbial contamination was studied. Biogenic amines were determined by fluorescence detection HPLC based on pre-column dansylchloride derivatization. The studied microbiological parameters included total plate count, coliform bacteria count and psychrotrophic bacteria count. Concentrations of biogenic amines (putrescine, cadaverine, spermidine, spermine, tyramine, histamine and tryptamine) determined in the muscle tissue of hybrids of individual lines varied depending on temperature and storage period. All four lines showed a significant increase ($P < 0.01$) in putrescine, cadaverine, spermidine, spermine and tyramine concentrations over 7-day storage at 3 ± 2°C (mean values 9.7 ± 9.5, 3.4 ± 4.2, 10.1 ± 13.4, 6.3 ± 2.1 and 26.2 ± 4.7 mg/kg, respectively). No histamine was detected throughout the storage period. A health-threatening level of histamine was detected in samples stored at 24 ± 1°C for 2 days, accompanied by marked sensory changes. The highest mean value was 333.0 ± 100.0 mg/kg. Higher levels reaching significance ($P < 0.01$) compared with fresh fish were determined for putrescine (91.3 ± 54.6 mg/kg), cadaverine (213.0 ± 83.7 mg/kg), and tyramine (138.0 ± 45.1 mg/kg). In samples stored at –18 ± 1°C for 3 months, the content of biogenic amines did not differ at the level of significance from the values determined in the fresh muscle tissue. Putrescine and cadaverine levels were the best correlates of growing bacteria count. Significant differences between individual hybrid lines of carp were detected at none of the storage temperatures, neither between individual biogenic amines nor between the microbiological indicators determined.

Keywords: biogenic amines; freshwater fish; storage; HPLC; microbiological quality

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Biogenic amines (BA) are basic organic compounds whose presence in food has been given relatively a lot of attention. Low concentrations of BA are a natural characteristic of a number of foodstuffs where they are present as natural metabolic products or intermediates. They are formed most often by relevant amino acid decarboxylation, in enzymatic reactions of tissue or microbial nature (Kalač and Křížek, 2002; Velíšek, 2002).

Higher BA concentrations can be found in fermented foodstuffs such as cheese, durable sausages, beer, wine, sauerkraut, etc. (Velíšek, 2002).

In some non-fermented foodstuffs, the presence and content of biogenic amines are primarily indicators of the quality of original material/foodstuff and the standard of the hygiene chain, especially in the storage process. Besides a number of other foodstuffs, this is true especially of fish and fish products (Kalač and Křížek, 2002).

The spectrum and content of individual biogenic amines found in different fish species vary. Important endogenous factors affecting the BA content in fish meat (just like in other meat) are availability and amount of free amino acids, pH, ionic strength, individual nutrient levels, content of inhibitory substances, etc. (Ruiz-Capillas and Jiménez-Colmenero, 2004). As far as exogenous factors are concerned, hygiene and production practice standards must be mentioned as the most important one, with emphasis on the initial microbial contamination of raw meat and temperature conditions in the whole hygiene chain.

Concentrations of biogenic amines in fresh fish are very low, their content increasing in the course of storage (Veciana-Nogués et al., 1997). Histamine and tyramine represent the highest toxicological risk. Concentrations of histamine in the muscle tissue of scombroid fish (e.g. mackerel, tuna, herring and sardine) depend on free histidine concentrations (Kalač and Křížek, 2002). Histidine content in the meat of different fish species varies to a great extent and cannot be used as the only indicator of fish meat quality (Mietz and Karmas, 1978; Suhaj and Kováč, 1996). Some authors (Mietz and Karmas, 1978) used the so-called biogenic amine index (BAI) as a qualitative criterion for fish quality assessment. This criterion is based on the established fact that the content of PUT, CAD and HIM in the meat of some sea animal species increases as the meat decays while SPD and SPM concentrations decrease. The applicability of this indicator however seems to be very limited and the index cannot be applied on a general basis.

Determining the content of biogenic amines in carp, Křížek et al. (2002) found BAI inappropriate as a criterion of meat quality assessment. They regarded the critical limit proposed by Mietz and Karmas (1978), 10 mg/kg, as too high for freshwater fish. To evaluate the quality of common carp, they used PUT content and summary PUT and CAD level, these concentrations being the best correlates of sensory properties in carp meat. They recommended PUT and/or PUT and CAD summary level as an alternative criterion of meat quality assessment. The above-quoted study suggests that biogenic amine formation in fish is species-specific and no ultimate interpretation of the results of determination can be applied. The growing interest in determination of individual biogenic amines and their concentrations in foodstuffs is due to a growing understanding of their biological effects on human health. Although biogenic amines are indispensable for humans, they may have psychoactive and vasoactive effects in high concentrations. The most conspicuous symptoms of consumption of high doses of biogenic amines are vomiting, respiratory difficulties, perspiration, palpitation, hypo- or hypertension (histamine) and migraine (2-phenylethylamine, tyramine) (Smělá et al., 2004). When their toxic effect is evaluated, we should also consider not only the presence of a specific amine but also other factors such as the amount of consumed food, the presence of other toxic substances (medicaments, alcohol), or degradation ability of the organism with respect to biogenic amines, etc. For this reason determination of toxicity of biogenic amines is beset with problems (Křížek et al., 2004).

The current Czech legislation (Decree, 2004), namely Directive No. 305/2004 Coll. as amended, sets down the admissible limit for histamine only, at 100 mg/kg (this limit may be surpassed by no more than 100% in 2 samples out of 9).

There is a number of studies dealing with the formation of biogenic amines in saltwater fish (Mietz and Karmas, 1978; Veciana-Nogués et al., 1997; Flick et al., 2001; Du et al., 2002; Kim et al., 2002). Published data on the formation of biogenic amines in freshwater fish is much scarcer (Ruiz-Capillas and Moral, 2001; Křížek et al., 2002, 2004). Some studies show that differences in the formation of biogenic amines in muscle tissues of different fish species have been confirmed, and

that intra-species differences in the dynamics of their formation must also be considered (Křížek and Kalač, 1998).

The goal of the present study is to establish potential differences in the dynamics of formation of biogenic amines in four hybrid lines of carp (*Cyprinus carpio*) with respect to the bacterial contamination of fish and storage conditions (3 ± 2°C; 24 ± 1°C and −18 ± 1°C). The determination of selected biogenic amines was based on implementation and validation of pre-column dansylchloride derivatization HPLC.

MATERIAL AND METHODS

Material

240 samples were taken from 40 recently killed specimens of carp (*Cyprinus carpio*) hybrids from four different lines living in Pohořelice carp ponds. These lines were obtained by hybridization of females of the Přerov fully scaled carp (PFSC) and male fish of other breeds – Ropsha scaled carp (Rop) and Northern mirror carp (M72). The resulting lines were as follows: PFSC for pure line production (referred to as line 1), PFSC × M72 (referred to as line 2), PFSC × Rop (referred to as line 3). The control was a mirror hybrid of Hungarian and Northern mirror carp (M2 × M72), which is used as a control line in common carp hybrid yield tests.

Each of the lines was represented by 10 samples. The fish were caught in a single pond at the same time. The fish meat was divided into 6 portions to be stored at various temperatures. Samples stored at 3 ± 2°C were analysed immediately after the killing (time 0), on days 2, 4 and 7; samples stored at 24 ± 1°C were analysed on day 2 and samples stored at −18 ± 1°C were analysed after 3 months of storage.

Methods

Biogenic amines (tyramine (TYM), histamine (HIM), putrescine (PUT), cadaverine (CAD), spermine (SPM), spermidine (SPD), and tryptamine (TRM)) were determined by pre-column dansylchloride derivatization HPLC as described by Paulsen et al. (1997).

Concentrations of biogenic amines were determined by HPLC using an Alliance 2695 liquid chromatofigure (Waters, USA) with a 2475 fluorescence detector and a PDA 2 996 detector. Separation was performed using a Polaris C18 column (Varian, USA) with reversion phase 4.6 × 150 mm and stationary phase grain size 3 µm, the column temperature being 35°C. Amine dansylderivatives were quantified by the external standard method using Empower software (Waters, USA).

Microbial contamination was established by determining total plate count (TPC), coliform bacteria count (CBC) and psychrotrophic bacteria count (PBC): 5 grams of fish muscle tissue were homog-

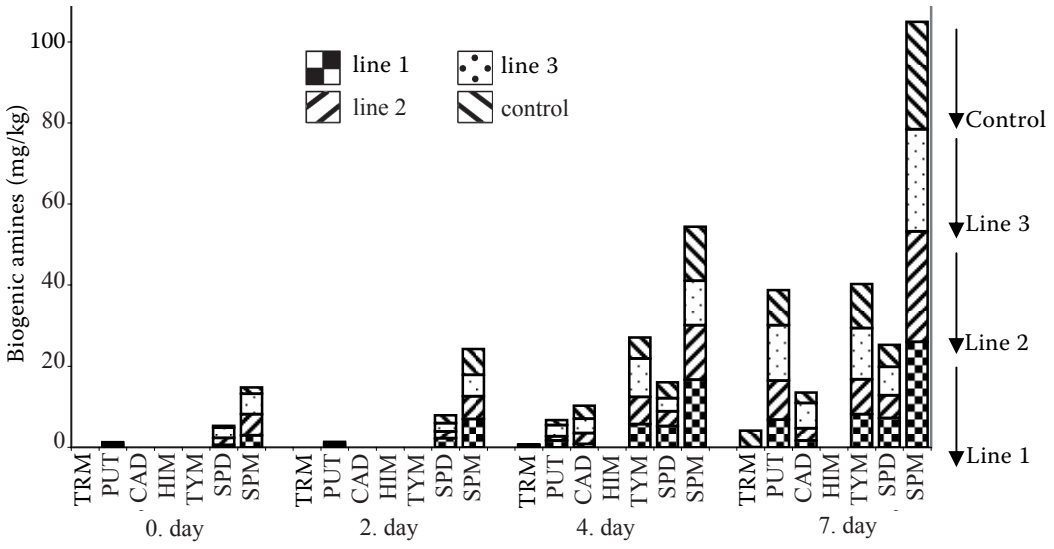

Figure 1. Levels of biogenic amines in mg/kg in the muscle tissue of carp of individual lines stored at 3 ± 2°C (plotted one above another to make the figure as clear as possible; the levels are not to be considered cumulatively)

Table 1. Levels of biogenic amines in the muscle tissue of carp stored at 3 ± 2°C

Amine (mg/g)	Days of storage											
	0			2			4			7		
	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.
TRM	ND	ND	ND	ND	ND	ND	0.2	ND	1.3	2.3	ND	90.8
PUT	0.3	ND	1.0	0.3	ND	2.9	1.8	ND	7.6	9.7	ND	40.9
CAD	ND	ND	ND	ND	ND	ND	2.6	ND	13.0	3.4	ND	16.7
HIM	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
TYM	ND	ND	ND	ND	ND	ND	6.8	ND	14.4	10.1	ND	32.1
SPD	1.3	0.1	3.6	2.0	0.4	3.6	4.0	1.4	7.4	6.3	4.4	13.9
SPM	3.7	0.7	12.7	6.1	1.2	11.1	13.6	7.8	28.4	26.2	19.0	43.2

Amine (mg/g)	SD	SD	SD	SD
TRM	0.4	0.4	1.7	14.4
PUT	0.5	2.0	4.1	9.5
CAD	ND	4.2	ND	4.2
HIM	ND	ND	ND	ND
TYM	ND	4.1	1.7	13.4
SPD	0.9	1.7	2.4	2.1
SPM	2.4	3.9	3.9	4.7

Min. = minimum value; Max. = maximum value; SD = standard deviation; ND = not detectable – the level is below the detection limit

enized with 45 ml of a diluent. Total microbial count was determined on PCA medium (HiMedia, India), aerobically, 72 hrs at 30°C, in accordance with the CSN ISO 4833 (2003) standard. Psychrotrophic bacteria count was determined on PCA medium (HiMedia, India), aerobically, 10 days at 6.5°C, in accordance with the CSN ISO 6730 (1996) standard. Coliform bacteria count was determined on VRBA medium (HiMedia, India), aerobically, 48 hrs at 30°C, in accordance with the CSN ISO 4832 (1995) standard.

The statistical evaluation of results was performed with the help of statistical and figureic software STAT plus (Matoušková et al., 1992). Prior to the proper testing the data was subjected to the Box-Cox transformation as its basic distribution did not meet the normality condition. The logarithm of TPC, CBC and PBC was taken. Then the data were subjected to Bartlett's homogeneity-of-variance test, analysis of variance and Scheffe's method for multiple contrasts was applied. Correlation and regression analysis were used in the second stage.

RESULTS AND DISCUSSION

Table 1 shows the mean concentrations of biogenic amines determined in the muscle tissue of carp from all 4 lines stored at 3 ± 2°C and the basic quantitative statistical characteristics. The amounts of biogenic amines for the individual lines throughout the storage at 3 ± 2°C are shown in Figure 1. In the control line of the above-named BA only putrescine, spermidine and spermine were detected at the start of the storage period, the mean concentrations being 0.3, 0.5 and 1.6 mg/kg respectively. During the storage a significant increase ($P < 0.01$) in putrescine, cadaverine, spermidine and tyramine concentrations occurred; only putrescine, spermidine and spermine were detected after 2 days of storage and tryptamine and tyramine joined the list after 4 days of storage. The concentrations after 7 days of cold storage were 8.7 mg/kg for putrescine, 2.6 mg/kg for cadaverine, 5.4 mg/kg for spermidine, 26.5 mg/kg for spermine and 10.9 mg/kg for tyramine. Histamine was not detected even after 7 days of storage.

Biogenic amine levels for lines 1, 2 and 3 stored at 3 ± 2°C showed a similar trend like the control line. At the start of storage also only putrescine, spermidine and spermine were detected. The respective mean concentrations of all these three amines were higher for lines 2 and 3 (0.5, 1.7 and 5.2 mg/kg for line 2 and 0.4, 2.6 and 5.1 mg/kg for line 3). The differences between the control group and the individual lines did not however reach the level of significance. The mean concentrations of spermidine and spermine in line 1 were higher than those of the control line; putrescine was the only amine whose concentration

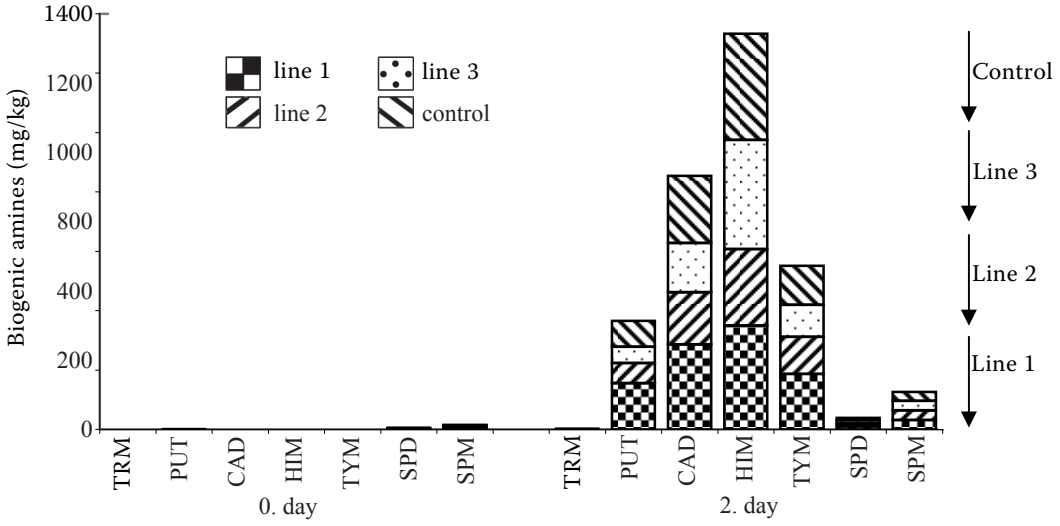

Figure 2. Levels of biogenic amines in mg/kg in the muscle tissue of carp of individual lines stored at 24 ± 1°C (plotted one above another to make the figure as clear as possible; the levels are not to be considered cumulatively)

was lower (0.1 mg/kg). The difference was not significant either.

These three groups showed a similar trend during storage like the control one. A significant ($P < 0.01$) increase in putrescine, cadaverine, spermidine, spermine and tyramine concentrations occurred over the storage period. At the end of storage, line 2 and line 3 showed higher putrescine, cadaverine and spermidine levels than the control group (9.7, 3.0 and 5.6 mg/kg respectively for line 2 and 13.6, 6.2 and 7.1 respectively for line 3), but the difference contrasting the values with the control group was not significant. Putrescine and cadaverine concentrations in line 1 were lower compared with the control group (6.8 and 1.7 mg/kg respectively) while the content of spermidine was higher (7.2 mg/kg). As far as tyramine is concerned, the concentrations detected in lines 1 and 2 were lower compared with the control group (8.2 and 8.6 mg/kg, respectively) while the concentration detected in line 3 was higher (12.7 mg/kg). This difference was not, however, significant either. Histamine was detected in none of the lines kept in cold storage; tryptamine was detected only in line 3 (9.1 mg/kg). Similar results were obtained by Křížek et al. (2004), who did not detect histamine after 7 days of cold storage either.

Figure 2 shows the amount of biogenic amines in samples of the individual lines stored at 24 ± 1°C.

Table 2. Levels of biogenic amines in the muscle tissue of carp stored at 24 ± 1°C

Amine (mg/kg)	Days of storage							
	0				2			
	Mean	Min.	Max.	SD	Mean	Min.	Max.	SD
TRM	ND	ND	ND	ND	0.4	ND	4.5	0.9
PUT	0.3	ND	1.0	0.3	91.3	15.2	238.0	54.6
CAD	ND	ND	ND	ND	213.5	96.4	376.9	83.7
HIM	ND	ND	ND	ND	333.0	139.0	660.0	100.3
TYM	ND	ND	ND	ND	137.0	77.9	244.0	45.1
SPD	1.3	0.1	3.6	1.2	9.7	3.7	15.1	1.7
SPM	3.7	0.7	12.7	2.7	31.5	25.2	46.9	3.8

Min. = minimum value; Max. = maximum value; SD = standard deviation; ND = not detectable – the level is below the detection limit

The highest concentration of all BA was that of histamine detected in samples of the control line (357.9 mg/kg). Other amines (putrescine, cadaverine, tyramine, spermidine and spermine) also showed a highly significant ($P < 0.01$) increase during this storage, the respective concentrations being 87.4 mg/kg, 226.0 mg/kg, 132.0 mg/kg, 8.9 mg/kg and 30.0 mg/kg. The detected concentration of tryptamine was 0.9 mg/kg.

Samples of lines 1, 2 and 3 stored at this temperature showed a similar significant increase in biogenic amines. The highest concentrations in all lines were those of histamine (349.0 mg/kg for line 1, 258.0 mg/kg for line 2 and 368.0 mg/kg for line 3). Line 1 had higher concentrations of putrescine, cadaverine and tyramine (156.0, 285.0 and 187.0 mg/kg) compared with the control line while the concentrations in lines 2 and 3 were lower (67.6, 176.0 and 125.0 mg/kg respectively for line 2 and 54.7, 165.0 and 107.0 mg/kg respectively for line 3). The respective levels of tryptamine detected in lines 1 and 2 were 176.0 and 125.0 mg/kg while no tryptamine was detected in line 3. Both spermidine and spermine were higher in all three lines compared with the control one (spermidine 9.5, 10.2 and 10.2 mg/kg respectively and spermine 30.9, 33.2 and 31.9 mg/kg, respectively). With this type of storage the differences between individual lines and the control one or just between individual lines were not significant either.

Table 2 sums up the results and basic quantitative statistical characteristics of biogenic amines in the muscle tissue of carp in all four lines stored at 24 ± 1°C. The highest concentrations in all four lines were those of histamine (mean value 333.0 mg/kg), the lowest were those of tryptamine (0.4 mg/kg), which is in line with the results reported by Křížek et al. (2004). Analysing samples of fish meat stored at 15°C, the lowest concentrations detected by these authors were also those of tryptamine, while the highest concentrations observed by them were those of cadaverine, as early as after 2 days of storage. In the same study it was putrescine levels, or rather summary putrescine plus cadaverine levels that best corresponded with negative sensory qualities of fish meat.

The increase in the content of biogenic amines in samples stored at –18 ± 1°C for 3 months was similar to that observed in samples stored at 3 ± 2°C (Figure 3). The results and basic quantitative statistical characteristics of biogenic amine determination in carp meat samples of all four lines stored at this temperature are summed up in Table 3. Just like with the control group, only putrescine, spermidine and spermine were detected in the experimental lines after 3 months of storage. Their content did not differ at the level of significance from the values found in fresh muscle tissue. Compared with the control group, spermidine levels were higher in line 1 (4.4 mg/kg) and lower in lines 2 and 3 (3.1 and 3.5 mg/kg). Spermine was higher in all three lines (7.4, 37.0 and 6.7 mg/kg respectively). Compared with the control line putrescine levels were higher in lines 2 and 3 (0.4 and 0.4 mg/kg, respectively). Lower putrescine concentrations were found in line 1

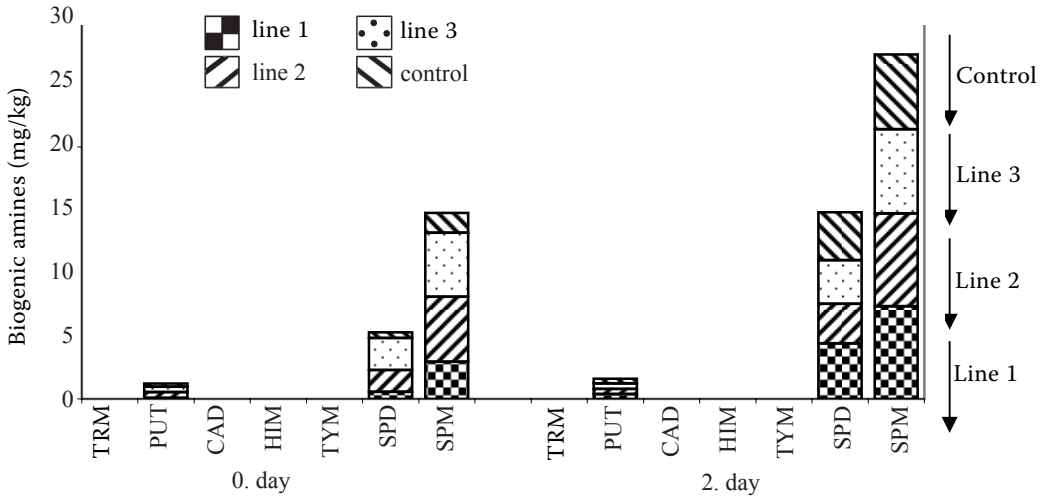

Figure 3. Levels of biogenic amines in mg/kg in the muscle tissue of carp of individual lines stored at –18 ± 1°C (plotted one above another to make the figure as clear as possible; the levels are not to be considered cumulatively)

Table 3. Levels of biogenic amines in the muscle tissue of carp stored at –18 ± 1°C

Amine (mg/kg)	Months of storage							
	0				3			
	Mean	Min.	Max.	SD	Mean	Min.	Max.	SD
TRM	ND	ND	ND	ND	ND	ND	ND	ND
PUT	0.3	ND	1.0	0.3	0.4	0.2	0.7	0.1
CAD	ND	ND	ND	ND	ND	ND	ND	ND
HIM	ND	ND	ND	ND	ND	ND	ND	ND
TYM	ND	ND	ND	ND	ND	ND	ND	ND
SPD	1.3	0.1	3.6	1.2	3.7	1.9	5.8	1.0
SPM	3.7	0.7	12.7	2.7	6.8	2.4	14.8	2.1

Min. = minimum value; Max. = maximum value; SD = standard deviation; ND = not detectable – the level is below the detection limit

(0.4 mg/kg). The differences were at the level of significance in none of the cases.

Summing up the results of determining the content of biogenic amines in all four lines we may say that in none of the cases were any significant differences in the formation of biogenic amines between the control group and the hybrid experimental lines proved. Histamine, regarded as highly problematic in saltwater fish as far as food certification is concerned, is no serious consumer health risk in freshwater fish. Its amount is low compared with saltwater fish and the development of toxic levels is preceded by diminished sensory quality. Other authors also arrived at the same conclusions about biogenic amines in freshwater fish (Ruiz-Capillas and Moral, 2001; Křížek et al., 2002, 2004, etc.). As far as biogenic amines are concerned, if prescribed sanitary and production practices, especially recommended storage conditions, are adhered to, the hybrid lines tested by us are a safe foodstuff.

A summary of the results of the microbiological analysis of samples of all four lines kept in cold storage at low temperatures is shown in Figure 4. The muscle tissue of freshly caught fish contains very low numbers of microorganisms – these are present in varying numbers especially on the gills, in the mucus on the surface of the body and in the intestine. Depending on the used technology and sanitary standard of fish meat processing and storage conditions, the contamination of muscle tissue and microorganism propagation occur.

The results of microbiological analysis evidenced a correlation between the numbers of bacteria of some groups under investigation and amounts of biogenic amines. The differences in numbers of

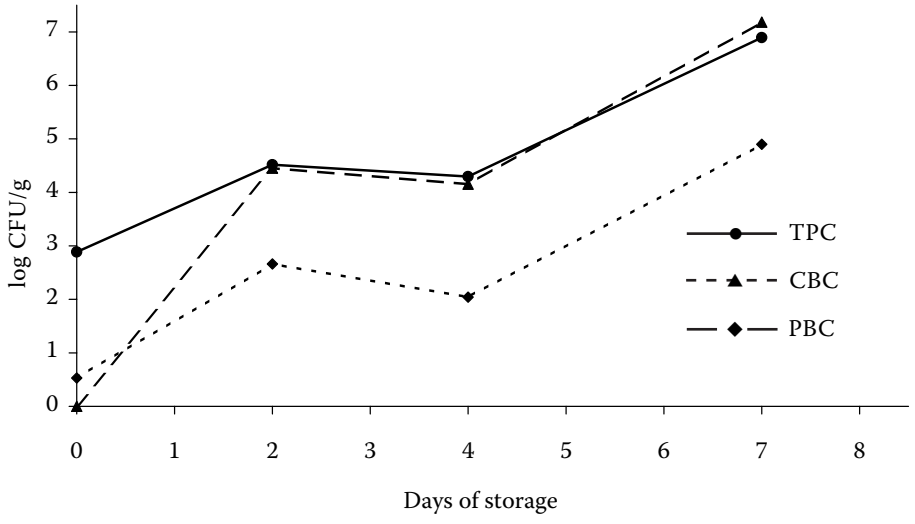

Figure 4. Mean bacteria counts (TPC, CBC, PBC) in meat of carp of four lines (n = 40) stored at 3 ± 2°C

Table 4. Correlation coefficients for log 10 CFU and individual biogenic amines in storage at 3 ± 2°C

Amine	TPC		CBC		PBC	
	4 days	7 days	4 days	7 days	4 days	7 days
TRM	0.086	0.098	0.078	0.268	0	0.075
PUT	0.226	0.554**	0.373*	0.469**	0.510**	0.547**
CAD	0.471**	0.482**	0.469**	0.607**	0.548**	0.441**
HIM	0	0	0	0	0	0
TYM	0.083	0.491**	0.335	0.353	0.207	0.459**
SPD	–0.484**	–0.042	–0.610**	–0.103	–0.427*	0.014
SPM	–0.359*	0.055	–0.288	0.093	–0.349	0.043
P + C	0.458**	0.572**	0.512**	0.550**	0.626**	0.553**

*$P < 0.05$; **$P < 0.01$; P + C = PUT + CAD; CFU = Colony Forming Units

microorganisms between individual groups of samples were not significant.

Average TPC and CBC in fish meat from fish of all four lines stored at 3 ± 2°C multiplied by the order of hundreds on average within 2 days and by another several hundred times within 7 days. In fish meat stored at 24 ± 1°C both TPC and CBC values multiplied by 10^4. In samples stored at –18 ± 1°C for 3 months, TPC values remained unchanged compared with values for fresh fish; CBC increased ten times on average compared with CBC of fresh fish. Psychrotrophic bacteria were detected in fresh fish meat in none of the lines. They were however detected after 2-day storage at 3 ± 2°C, the average values approximating 10^4 CFU/g, and the count multiplied by another 10^3 after 7 days. CBC of fish meat stored for 2 days at 24 ± 1°C increased roughly to 10^5 CFU/g. The psychrotrophic bacteria count in fish meat stored for 3 months at –18 ± 1°C increased by the order of hundreds. Similar results were obtained by Lukášová et al. (2003), who stored samples of common carp at 3°C. In these samples, the mesophilic bacteria count multiplied by the order of 10^4 within 7 days of storage and coliform bacteria count multiplied by the order of 10^3. Psychrotrophic bacteria were not detected in fresh meat samples; the count increased by the order of 10^6 within 7 days of storage. Our results are in agreement with results of Křížek et al. (2004), who concluded that the formation of biogenic amines was related to mesophilic rather than psychrotrophic bacteria.

As far as the samples stored at 3 ± 2°C are concerned, results of microbiological analysis correlated with putrescine and cadaverine at the level of high significance ($P < 0.01$). Spermidine correlated at the level of high significance ($P < 0.01$) with CBC and TPC and spermidine at the level of significance ($P < 0.05$) with PBC. With these two amines only, the correlation coefficients had negative values, i.e. the amount of these biogenic amines decreased as the overall bacteria count increased (Table 4).

CONCLUSION

Bacteria counts of stored fish meat depend on the initial contamination of the material as well as on storage temperature and period. Biogenic amines were already present in fresh meat and their content increased during storage depending on temperature. It was especially concentrations of putrescine, cadaverine, spermidine, spermine and tyramine that increased. Biogenic amine levels correlated with growing bacteria counts, especially mesophilic bacteria count. Levels of histamine relevant in terms of health considerations (333.0 mg/kg) were detected in samples showing symptoms of advanced meat decay, especially in samples stored at 24 ± 1°C for 2 days. At lower storage temperatures conforming to legislation requirements, the content of biogenic amines was in line with norms set by current legislation. The content of biogenic amines in meat stored in cold storage did not differ from the values detected in fresh muscle tissue.

It is the summary level of putrescine and cadaverine that can be regarded as the most objective indicator of meat quality, whose content best correlated with growing bacteria counts. As long as prescribed storage temperatures were adhered to, the amount of newly formed biogenic amines was low. As far as the three experimental hybrid lines

of common carp are concerned, the results of our study allow us to conclude that no differences in biogenic amine formation were recorded.

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