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Formation of selected biogenic amines in carp meat

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Abstract: Changes in biogenic amine content in carp (*Cyprinus carpio*) muscle were studied. Fish halves and minced fish meat were stored at 3 and 15 °C. Both the temperature of storage and the type of meat processing had statistically important effects on the amine content. In another set of experiments, temperature and the preservative effects of Purac at various concentrations were tested. Purac can extend the shelf-life of fish halves stored at 3 °C by about 5 days. Putrescine concentration is proposed as a chemical indicator of carp meat quality. Decomposition is apparent when the putrescine content in the meat exceeds 20 mg kg^{-1}.

Keywords: biogenic amines; putrescine; freshwater fish; quality changes

INTRODUCTION

Biogenic amines such as putrescine (PUT), cadaverine (CAD), spermidine (SPD), spermine (SPM), histamine (HI), tyramine (TY) and tryptamine (TR) are widely distributed in proteinaceous foods. Food quality has been linked to these amines in fermented or fresh foodstuffs. Amines are formed by decarboxylation of amino acids as a result of metabolic processes in plants and micro-organisms. In stored meat, amines are generated by the action of spoilage bacteria decarboxylases.

There are two reasons for the determination of amines in foods: the first is their potential toxicity; the second is the possibility of using them as food quality markers. Biogenic amines at low concentrations are essential for many physiological functions,[1] while ingestion of large amounts may result in health problems.[2]

Amine toxicity depends strongly on the individual efficiency of detoxication.[3] Normally, during the food intake process in the human gut, low amounts of biogenic amines are metabolised to physiologically less active degradation products. This detoxification system includes specific enzymes such as diamine oxidase (DAO). However, upon intake of high loads of biogenic amines in foods, the detoxification system is unable to eliminate these biogenic amines sufficiently. Moreover, in the case of insufficient DAO activity, caused for example by generic predisposition, gastrointestinal disease or inhibition of DAO activity due to secondary effects of medicines or alcohol, even low amounts of biogenic amines cannot be metabolised efficiently.[4] Some biogenic amines, eg histamine and tyramine, are considered as antinutritional compounds. For sensitive individuals they represent a health risk, especially when their effect is potentiated by other substances. Poisoning by histamine with its allergy-like symptoms is usually related to the consumption of scombroid fish such as tuna or mackerel[5,6] and is considered to be one of the commonest forms of food intoxication reported.[7]

Biogenic amines in fish can serve as indicators of decomposition. Mietz and Karmas[8] found that the HI content varied extensively with the fish species and they did not recommend using histamine determination for fish meat quality assessment. Instead they proposed the complex BAI criterion (see Table 9). Recently, this finding was confirmed by Mendes[9] in investigations of histamine formation in sardines and mackerel. No correlation between HI formation and the decline in organoleptic quality of sardines was found. Yamanaka *et al*[10] evaluated the decomposition of chum salmon (*Oncorhynchus keta*) and proposed CAD as the best indicator of spoilage. A good correlation was found between organoleptic evaluation and levels of PUT and CAD in skipjack tuna.[11] Veciana Nogués *et al*[5] proposed the use of an index calculated from the sum of the contents of HI, TY, PUT and CAD for tuna assessment. It can be concluded that the amines formed in the matrix depend on the fish species.

In contrast with the number of workers examining changes in biogenic amine content in sea fish,[10,12–14] freshwater fish have not been studied yet. The objective of the present work was to learn how the temperature of storage and the type of meat processing can affect biogenic amine formation in carp muscle.

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MATERIALS AND METHODS

Experimental

The experiment was divided into two parts. First we compared the kinetics of amine formation in fish halves and fish mince. Then we studied the kinetics of amine formation in portions of fish halves preserved by the addition of Purac.

Samples

Samples were obtained from freshly killed carp (*Cyprinus carpio*) of average body mass 2.3 kg (2.0–2.6 kg). The fish were drawn and, after decapitation and trimming away the tail, the body was cut into two halves (fillets with backbones). These halves, which represented the ordinary edible meat muscle, were washed in water, cut into pieces (approx 40 g) and used in the experiments.

(a) Fish halves were further processed in a fish meat separator.
(b) Pieces of fish halves were treated with Purac.

Experiments were designed to study the kinetics of deterioration. Samples of fish halves and fish mince were analysed in duplicate every 24 h of storage up to the stage of apparent decomposition. The maximum times of storage were 4 and 13 days at temperatures of 15 and 3 °C respectively.

Purac treatment

Purac (PURAC Biochem, Gorinchem, The Netherlands) is a preservative agent based on lactic acid. Three different concentrations of lactic acid were used: 20, 30 and 50 g kg^{-1}. Samples of meat muscle were immersed in Purac solutions for 1 min. One portion was immersed in distilled water to serve as a control sample.

Sample storage and temperature conditions

Both kinds of sample, ie pieces of fish halves and minced fish meat from the separator, were stored in polyethylene bags. The wrapping was careful, but purposely not airtight, simulating normal conditions in households and protecting the sample from desiccation.

Experiments were conducted at 3 and 15 °C, and also at 25 °C when Purac was applied. A temperature of 3 °C is regarded as relatively safe for fish meat storage. In order to simulate situations where the temperature control fails temporarily, some samples were stored at 15 °C, while the temperature of 25 °C was chosen in order to test the action of Purac.

Analytical method

Biogenic amines (PUT, CAD, SPD, SPM, HI, TY and TR) were extracted from homogenised meat samples (40 g) with dilute perchloric acid (pa, 0.6 M; Fluka, Buchs, Switzerland). After filtration the volume was made up to 150 ml with perchloric acid. Centrifugation was not necessary, as carp meat has a relatively low content of fat. The amines were determined as the *N*-substituted benzamides, after derivatisation with benzoyl chloride, by micellar electrokinetic capillary chromatography using a capillary zone electrophoresis instrument. Duplicate analyses were carried out. The procedure has been described in detail by Křížek and Pelikánová.[15]

Apparatus

The TR-6 meat separator was from Nurle Oy (Helsinki, Finland). In the separator, meat is squeezed through holes into a cylinder, under pressure applied by a conveyor belt.

Samples were homogenised in either a commercial food hand-blender (Philips, Vienna, Austria) or an Ultra-Turrax T25 homogeniser (Ika Labortechnik, Staufen, Germany).

Analyses were carried out using a Spectraphoresis 2000 instrument, a fully automated system for capillary zone electrophoresis equipped with a multi-wavelength UV-VIS scanning detector (Thermo Separation Products, Fremont, CA, USA). Separations were achieved using a plain fused silica capillary of 43 cm total length (36 cm effective length to the detector) and 75 μm inner diameter (CElect FS75 CE column, Supelco, Bellefonte, PA, USA).

Data processing was performed using Spectacle and PC 1000 CE software v 3.0 (Thermo Separation Products).

Sensory tests

Sensory tests were only complementary to the main objective of this study—the determination of chemical changes in the meat 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. Table 1 specifies the three levels.

Statistical evaluation

Samples were prepared in duplicate and each sample was analysed twice. Samples included in the analysis of variance were replicated five times. Analysis of variance was carried out using basic statistical proce-

Table 1. Simple scheme for organoleptic evaluation of carp meat

	Quality (sensory score)		
	Good (1)	*Acceptable (2)*	*Poor (3)*
Odour	Meaty, neutral	Neutral, slightly spicy	Fishy, repulsive
Appearance (colour/texture)	White/tightly elastic	Greyish/solid	Grey/muddy

Table 2. Average amine contents (mg kg^{-1}) in carp meat (five replicate analyses) on second day of storage and *F* criteria for both factors (critical *F* value for five repetitions, *F*(crit) = 4.494). Factor A: type of processing (1 = fish halves; 2 = fish mince). Factor B: temperature of storage (1 = 3 °C; 2 = 15 °C)

Amine	*A(1) B(1)*	*A(2) B(1)*	*A(1) B(2)*	*A(2) B(2)*	F*(A)*	F*(B)*
PUT	2.30	4.14	17.2	33.2	7.277	69.84
CAD	1.75	4.17	20.0	215	486.2	654.4
SPD	11.1	8.81	9.20	4.35	8.041	6.378
SPM	6.23	12.3	8.55	8.43	13.81	0.948*
HI	0.28	0.49	0.84	179	829.9	836.4
TY	6.19	1.40	4.85	773	53.04	58.21
TR	0.48	0.80	0.87	3.49	8.812	9.696

* Subcritical value ($P < 0.05$).

dures implemented as macros in MS-Excel spreadsheets (Excel 2000, Microsoft Corp, Redmond, WA, USA). Fitting of curves describing the kinetics of amine formation was done using the mathematical software Maple V v 5.0 (Waterloo Maple Inc, Waterloo, Canada).

RESULTS AND DISCUSSION

Pieces of fish halves and fish mince

To test for differences in amine content in samples at the beginning of storage, the second day of storage was chosen for the analysis of variance. On the second day, five replicates of each kind of sample were analysed. The type of processing (factor A) was tested on two levels: 1-fish halves; 2-fish mince. The temperature of storage (factor B) was also tested on two levels: 1–3 °C; 2–15 °C. Results of the analysis of variance are given in Table 2.

The data in Table 2 reveal that both the temperature of storage and the type of processing had significant effects on the amine content in the early stage of storage. The only exception was SPM, where the influence of temperature was not significant. As expected, samples of fish mince and samples kept at 15 °C developed higher contents of amines. SPD, in accordance with Mietz and Karmas,[8] showed a decrease with the progress of decomposition at both temperatures.

For fish meat a close correspondence between stage of decomposition and sensory properties is very characteristic. In all our experiments (including those with Purac) the best correlation was observed between PUT content and flesh spoilage. Correlation coefficients ($P \leqslant 0.05$) between amine content and sensory levels were 0.939, 0.911, 0.699, 0.416, 0.356 and 0.272 for PUT, CAD, HI, TY, SPD and SPM respectively.

Samples of good quality (1) contained up to 10 mg kg^{-1} putrescine. For acceptable quality (2) we propose the range 10–20 mg kg^{-1}. In samples with PUT content above 20 mg kg^{-1}, decomposition was apparent (poor quality (3)). The kinetics of PUT formation is shown in Table 3. The kinetics of histamine formation was similar, though not as smooth as that of PUT, and an increase in HI content was apparent in samples of evidently bad quality (Table 4). The kinetics of formation of the other amines displayed similarly increasing trends, with the exception of SPD which showed a slight decrease with time (results not shown).

In contrast with Mietz and Karmas,[8] no decrease in SPM was observed, probably owing to the fact that those authors studied various kinds of sea species (salmon, rockfish, lobster, shrimp). The BAI criterion is based on the observation that the contents of PUT, CAD and HI increase while those of SPD and SPM decrease with the progress of decomposition of these kinds of fish. Use of the BAI criterion cannot be generally recommended.

As the kinetic curves of amine formation reveal a smooth change (usually an increase), they can be

Table 3. Kinetics of putrescine formation and sensory quality grades of carp meat: (a) putrescine concentration (mg kg^{-1}); (b) sensory score (1 = good, 2 = acceptable, 3 = poor)

	Day													
	0	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*
3°C														
Fish halves (a)	8.49	1.88	2.30	2.65	3.14	1.83	1.31	12.2	14.3	19.8	17.1	31.8	39.6	54.8
(b)	1	1	1	1	1	1	1	1	2	2	2	3	3	3
Minced meat (a)	1.39	1.84	4.14	9.89	9.04	12.0	24.9	11.7	36.5	49.3	69.9	96.9	125	183
(b)	1	1	1	1	2	2	3	3	3	3	3	3	3	3
15°C														
Fish halves (a)	8.49	4.84	17.2	77.7	86.6									
(b)	1	1	2	3	3									
Minced meat (a)	1.39	6.82	33.2	73.2	172									
(b)	1	1	3	3	3									

Table 4. Kinetics of histamine formation and sensory quality grades of carp meat: (a) histamine concentration (mg kg^{-1}); (b) sensory score (1=good, 2=acceptable, 3=poor)

	Day													
	0	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*
3°C														
Fish halves (a)	0.01	0.01	0.28	0.40	0.15	0.70	1.62	0.04	0.01	0.22	0.01	0.59	0.25	20.1
(b)	1	1	1	1	1	1	1	1	2	2	2	3	3	3
Minced meat (a)	0.01	0.01	0.49	0.01	0.32	0.66	0.59	0.06	10.1	13.9	28.3	46.6	110	205
(b)	1	1	1	1	2	2	3	3	3	3	3	3	3	3
15°C														
Fish halves (a)	0.01	0.59	0.84	31.9	42.1									
(b)	1	1	2	3	3									
Minced meat (a)	0.01	0.01	180	363	172									
(b)	1	1	3	3	3									

described by quadratic, cubic or exponential equations. By solving these three kinds of equation (calculations not shown) for the proposed three critical concentrations of PUT (10, 15 and 20 mg kg^{-1}), we obtained estimates of the respective critical times (days) when the given concentrations were reached. All three types of equation provided very similar results, as can be seen from the ranges in Table 5.

On comparing fish halves and minced meat kept at 3 °C, the critical concentration of PUT was reached 2–3 days earlier in the minced meat samples than in the halves. At 15 °C the decomposition processes were very fast and there was no apparent difference in the onset of critical PUT concentrations in the two types of sample. The temperature of storage played a key role in the kinetics of decomposition. At 3 °C the shelf-life was about 6 days for the fish halves and about 4 days for the minced meat. At 15 °C the decomposition was so fast that critical PUT concentrations were reached within 5–7 h regardless of the method of processing.

Application of Purac

The preservative effect of Purac was tested on pieces of carp meat as described above. Lactic acid, the substantial component of Purac, was added in defined concentrations to the samples. Another approach to preservation, using inoculation by lactic acid bacteria, was applied by Glatman *et al.* [16]

As it was not useful to prolong meat storage beyond the very bad sensory stage, the experiments with samples stored at 15 and 25 °C were stopped after 4 or even 3 days. Samples stored at 3 °C were examined for 14 days. From a toxicological point of view, biogenic amines in carp meat do not represent any hazard to consumers. Sensory signals precede the formation of toxic levels of histamine and tyramine—amines of the greatest toxicological importance. This is a big difference between scombrid fish meat and carp meat. The reason is probably that scombrid fish meat contains significant amounts of free histidine,[7] so its odour cannot be exclusively associated with the biogenic amine content. The fishy smell typical of the beginning of the decomposition process is a complex of the odours of many different substances.

While the toxicological aspect does not seem to be of critical importance in carp meat, the possibility of using amine concentration as a quality marker is more promising. By the third day of storage the differences in sample quality were usually conspicuous.

In Figs 1 and 2 we can see the dramatic effect of temperature on the putrescine and histamine contents on the third day of storage. Concentrations of these amines at 3 °C were negligible. On the other hand, it is seen that at 25 °C Purac had only a weak positive effect on the suppression of putrescine. The effect of Purac

Table 5. Days of storage of carp meat when given putrescine concentrations were reached, calculated as average of solutions of quadratic, cubic and exponential equations. The range of calculated results is shown in parentheses

	PUT concentration (mg kg^{-1})		
	10	*15*	*20*
Fish halves, 3 °C	6.3 (6.0–6.6)	7.7 (7.4–7.9)	8.7 (8.3–9.3)
Minced meat, 3 °C	4.3 (3.8–4.8)	5.2 (5.1–5.3)	5.8 (5.7–6.0)
Fish halves, 15 °C	0.2 (0.0–0.5)	0.5 (0.3–0.7)	0.8 (0.6–0.9)
Minced meat, 15 °C	0.3 (0.1–0.4)	0.6 (0.4–0.7)	0.8 (0.7–1.0)

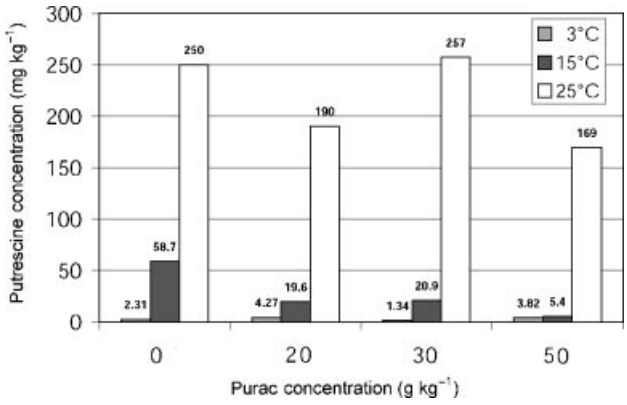

Figure 1. Effects of storage temperature and Purac concentration on putrescine concentration in carp meat after 3 days of storage.

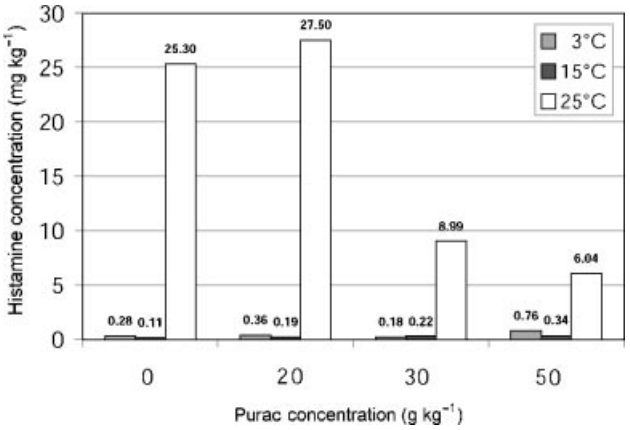

Figure 2. Effects of storage temperature and Purac concentration on histamine concentration in carp meat after 3 days of storage.

was more pronounced at 15 °C, where there was a decreasing trend in putrescine content linked with increasing Purac concentration (Fig 1). A greater influence of Purac at the highest temperature (25 °C) is apparent for histamine (Fig 2). We can also see relatively low histamine concentrations ($<50\,mg\,kg^{-1}$) in samples that were apparently at a bad sensory stage on the third day of storage. The effect of temperature was more distinct than that of Purac.

Putrescine showed the best correspondence between amine concentration and the sensory stage of the meat. Tables 6–8 show the kinetics of putrescine formation with respect to storage temperature and Purac concentration.

When we take into consideration the critical $20\,mg\,kg^{-1}$ level, it is seen that Purac extended storage times by about 5 days at 3 °C and about 1 day at 15 °C; at 25 °C the extension was difficult to assess, but it was less than 1 day. All the kinetic curves were fitted by the mathematical software Maple V, v 5.0 with the best correlation being given by exponential equations.

The kinetics of formation of CAD was almost the same as that of PUT. The onset of elevated concentrations of cadaverine was at nearly the same time as for putrescine.

Histamine and tyramine concentrations were generally low at 3 and 15 °C, typically below $10\,mg\,kg^{-1}$ without apparent increase, and bad sensory signals preceded the onset of formation of these amines, which in our opinion cannot be regarded as marking the beginning of decomposition. Similar results were obtained by Sato *et al*,[12] studying tuna fish, who showed that CAD was produced first, followed by HI. Spermidine and spermine in our samples were present in fairly low concentrations, fluctuating between 3 and $12\,mg\,kg^{-1}$. Spermidine showed a slightly decreasing trend with time. A similar SPD concentration decline was observed by Mietz and Karmas.[8] In contrast with their results, in our experiments a decline in concentration was not found for SPM. The low and fluctuating concentrations of both SPD and SPM make it difficult to link these amines with the description of the decomposition process. SPD and SPM are biosynthesised from putrescine.[17]

Table 7. Content of putrescine ($mg\,kg^{-1}$) in carp meat samples at 15 °C together with sensory evaluation (see Table 6 for definitions of P and S)

	Day				
	0	*1*	*2*	*3*	*4*
Control	2.0	2.0	16.0	58.7	162
S	1	1	2	3	3
P20	2.0	3.5	3.7	19.6	73.3
S	1	1	1	2	3
P30	2.0	3.0	2.1	20.9	55.0
S	1	1	1	1	3
P50	2.0	1.4	9.5	5.4	45.7
S	1	1	1	1	3

Table 8. Content of putrescine ($mg\,kg^{-1}$) in carp meat samples at 25 °C together with sensory evaluation (see Table 6 for definitions of P and S)

	Day			
	0	*1*	*2*	*3*
Control	2.0	9.7	123	250
S	1	2	3	3
P20	2.0	2.3	23.6	190
S	1	1	3	3
P30	2.0	2.4	72.3	257
S	1	1	3	3
P50	2.0	2.9	89.7	169
S	1	1	3	3

Table 6. Content of putrescine ($mg\,kg^{-1}$) in carp meat samples at 3 °C together with sensory evaluation: P, concentration of Purac applied ($g\,kg^{-1}$); S, sensory score (see Table 1 for definitions)

	Day														
	0	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*
Control	2.0	0.9	0.8	2.3	10.2	7.5	0.5	4.1	16.1	27.4	21.4	43.8	89.7	65.2	131
S	1	1	1	1	1	1	1	1	2	3	3	3	3	3	3
P20	2.0	2.1	6.2	4.3	3.5	4.4	2.6	4.7	4.5	20.7	5.6	40.3	25.5	66.5	104
S	1	1	1	1	1	1	1	1	1	2	2	3	3	3	3
P30	2.0	1.5	3.8	1.3	7.3	3.0	11.0	1.8	1.3	2.2	3.2	8.1	6.4	35.3	65.0
S	1	1	1	1	1	1	1	1	1	1	1	1	1	3	3
P50	2.0	2.6	4.0	3.8	12.8	2.1	3.5	2.3	1.1	3.7	2.7	10.3	10.6	27.0	54.4
S	1	1	1	1	1	1	1	1	1	1	1	1	2	3	3

Table 9. Comparison of sensory scores and amine concentrations (mg kg^{-1}) in carp meat. BAI = (PUT + CAD + HI)/(1 + SPD + SPM)

	Sensory score		
	1	*2*	*3*
PUT	<10	10–20	>20
CAD[a]	<10	10–25	>25
PUT + CAD[a]	<20	20–45	>45
BAI[a]	<1.5	1.5–3.2	>3.2

[a] Values calculated from the kinetics in relation to the proposed PUT levels.

When calculating the BAI quality criterion according to Mietz and Karmas[8] (Table 9), we found small delays in onset owing to histamine being involved in this criterion. In accordance with Veciana Nogués *et al*,[5] we consider the critical limit of BAI (10 mg kg^{-1}) to be high for carp meat. In Table 9 we propose the above-described critical contents of PUT in carp meat (<10, 10–20 and >20 mg kg^{-1} for good (1), acceptable (2) and poor (3) quality respectively). The respective CAD, PUT + CAD and BAI levels were calculated from the mathematically processed kinetics of amine formation in relation to the given PUT limits. The sum of putrescine and cadaverine seems to be a very promising criterion for carp meat, because both these amines have similar smooth kinetics, slightly preceding the sensory signals. A more complex quality index was proposed by Veciana Nogués *et al*[5] for tuna fish, calculated as the sum of the contents of PUT, CAD, HI and TY. As the elevation of HI and TY concentrations in carp meat occurs in stages of apparent matrix decomposition, we do not consider the incorporation of these two amines into the quality indicator for carp meat to be necessary.

CONCLUSION

Biogenic amines in stored carp meat do not represent any health hazard for individuals, because sensory signals precede the formation of toxic levels of the most toxic amine, namely histamine. The contents of the most problematic amines, ie histamine and tyramine, in carp meat are lower than in mackerel or tuna fish. Putrescine seems to be a good quality marker, as its concentration corresponds with the sensory signals of the samples. Temperature has a dominant effect on biogenic amine formation. The effect of Purac is clear, but it cannot counteract the influence of temperature. However, short-time failures of refrigeration systems might be overcome by Purac addition. On the other hand, the application of Purac is problematic, because the meat loses its natural appearance.

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