

BIOGENIC AMINES CONTENT OF ROMANIAN MARKET MACKEREL

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Abstract

*In Romania we eat only imported mackerel (*Scomber scombrus*) because we do not have this kind of fish locally. In our paper we studied samples of frozen and smoked mackerel from Romanian market terms of biogenic amines content. The purpose was to find if the samples had low or high quantities of biogenic amines, because it is well known that amines such as: histamine and tyramine when exceeding some values are bad for human health, especially in scombroid fishes. Biogenic amines from mackerel samples were determined by HPLC (high pressure liquid chromatography) and the amines traced were: tryptamine, β -phenylethylamine, putrescine, cadaverine, histamine, serotonin, tyramine, spermine and spermidine. In frozen mackerel samples we detected small amounts of histamine, cadaverine, spermidine and putrescin. Only one sample of the frozen mackerel showed clearly the incipient state of spoilage. The biogenic amines from smoked mackerel samples were higher than those found in frozen mackerel. In smoked samples we have not detected serotonin, and only one sample had phenylethylamine. This sample also showed an incipient state of spoilage. Histamine and tyramine values were higher in smoked mackerel samples than in the frozen ones. The quantities found of those two amines in the analyzed samples do not cause health problems to people health.*

Keywords: *spoilage, fish meat, histamine, tyramine, scombroid poison, biogenic amines.*

Introduction

Scombroid fish poisoning (scombrotosis, scombroid ichthyotoxicosis) is a food-related illness typically associated with the consumption of fish. Most of the published literature suggests that symptoms are related to the ingestion of biogenic amines, especially histamine; others, like putrescine and cadaverine, may potentate toxicity. Although scombroid poisoning is more common in nations with a warm water fishing industry, the illness is worldwide in scope. The most commonly implicated fish species are scombroid dark meat fish (eg, tuna, mackerel, skipjack, bonito, and marlin) and nonscombroid species, such as mahi-mahi (dolphinfish), amber jack, sardine, yellowtail, herring,

and bluefish. Although rare, cases of whitefish scombrotosis also have been reported. [1]

Cadaverine, putrescine and histamine can be produced postmortem from decarboxilation of correspondant free aminoacids in mackerel: histidine-histamine, ornithine-putrescine, lysine-cadaverine. Those are biogenic amines that influence negatively the consumer's health and sensorial characteristics of mackerel. Other amines that can be found in mackerel are: tyramine, tryptamine, 2-phenylethylamine. Also polyamines: spermine and spermidine can be found in mackerel. The decarboxilation process takes place in two biochemical processes: -by endogenous decarboxylase enzymes naturally occurring in mackerel and

-by exogenous enzymes released by microorganisms associated with the mackerel.

Hystamine is the most studied biogenic amine in mackerel meat. This amine is associated with the illness called scombrototoxicosis. The content of histamine must be higher than 300 mg, otherwise the intoxication do not occur [2]. In fresh mackerel, the content of biogenic amines varies in function of the anatomic part studied, of muscle type and of processing after catch. Intestinal wall samples contain high amine levels than muscles [3].

In red muscle mackerel histamine and histidine were produced in larger amount as compared to white muscle.

The effect of evisceration on biogenic amines production is inconsistently reported in literature. The rates of cadaverine and putrescine production can be ranked as follows: whole ungutted fish > fillets from whole ungutted fish and fillets from gutted fish > whole gutted fish [4]. This generalized description is heavily dependent on the extrinsic factors such as harvesting method and procedures for transportation, processing and retailing.

After hot smoking of frozen mackerel the study made by Zotos et al. [5] showed that a significant increase in histamine formation in previously frozen mackerel was solely due to temperature and reduction in smoking process. So, it was demonstrated that is very important to control temperature and time in the smoking process.

Our objectives were to find out the biogenic amines content of some market samples of mackerel (*Scomber scombrus*) and if those values found represent a threat to consumer's health.

Materials and methods

We went to a hypermarket and bought frozen mackerel, with head, guts and tail,

packaged in plastic bag and gutted smoked mackerel headless, gutted and without tail, packaged individually in vacuum. Frozen mackerel were put in refrigerated box and then transported to our laboratory where it was analyzed. The smoked mackerel was transported normally, with no special preservation techniques. All the samples were from the same producer.

After we had the samples in our laboratory, we checked the shelf life term and all the samples were at the second third period of validity term.

The samples processing was made as follows: we cut transversally three pieces of mackerel, first near the head, the second in the fish middle and the third near to fish tail. The width of the fish cut was of 2 cm. After that we deboned the fish and, if necessary we eliminated the guts.

The measurement of biogenic amines content using high performance liquid chromatography was performed according to the method proposed by Food Research Institute from Helsinki, Finland [6]. All the reagents used were analytic pure, for HPLC use. The water used was deionised. The necessary reagents were purchased from the Merck and Sigma-Aldrich companies. Installations and equipment used for biogenic amine determination: Philips 7768 food processor, homogenisation device 7011S, Kern 770-60 analytical balance, Silent CrusherM homogenisation device, centrifuge EBA 21, filter paper for quick filtering with 55 mm diameter, syringe filters with porosity of 0,45 μm and 13 mm diameter, Heidolph REAX control agitator, ultrasonic water tank Aquawave TM, incubator BMT INCUCCELL 55, water deionising system EASY pure RoDi, filtering assembly with vacuum pump. The device for the HPLC determination was a liquid chromatograph model SURVEYOR produced by Thermo Electron company, configured with detector model PDA PLUS DETECTOR, auto-sampler model AUTOSAMPLER

PLUS, pump model LC PUMP PLUS and detector UV-VIS. Chromatography column of type BDS Hipersyl C18. The biogenic amines quantification: quantitative measurement was performed depending on the internal standard using peaks for each biogenic amine. The 254nm wavelength absorbance was measured and the resulted peaks were integrated with CromQuest software. The concentration of each biogenic amine was expressed in mg/kg.

The method principle is as follows:

- Bioactive amines are extracted from a homogenized sample with diluted perchloric acid;
- An aliquot of the extract is derivatised with dansyl chloride reagent;
- Separation and quantification of dansylated amines is performed by reversed phase liquid chromatography with ultraviolet detection at 254 nm.

The values obtained for biogenic amines content for each sample were done in triplicates.

Our determinations refer to the following amines: tryptamine, β -phenylethylamine, putrescine, cadaverine, histamine, serotonin, tyramine, spermine and spermidine.

Results and discussion

In order to know exactly if Romanian market mackerel samples have biogenic amines content and to what extent we analyzed frozen mackerel and smoked mackerel, and the data obtained are shown in table 1 and table 2.

In the case of table 1 it is clear that phenylethylamine and serotonin were below the detection concentration in all the samples analyzed.

Table 1.

Biogenic amines content of frozen mackerel samples

Sample number	Biogenic amines (mg/kg)								
	TRYPT	FEN	PUT	CAD	HIST	SER	TYR	SPD	SPM
1	Nd	Nd	3.7	Nd	1.1	Nd	Nd	1.3	0
2	Nd	Nd	6.2	Nd	Nd	Nd	12.1	0	Nd
3	Nd	Nd	9.6	Nd	Nd	Nd	30.5	1.8	Nd
4	Nd	Nd	Nd	Nd	Nd	Nd	7.4	0	Nd
5	Nd	Nd	7.5	Nd	Nd	Nd	Nd	1.5	Nd
6	0	Nd	2.2	1.3	1.7	Nd	42.6	2.3	Nd
7	Nd	Nd	5	Nd	2.6	Nd	15.5	1	0
8	0	Nd	Nd	0	Nd	Nd	Nd	0	0
9	0	Nd	Nd	Nd	0	Nd	7.7	2.1	Nd
10	Nd	Nd	9	0	2.4	Nd	13.9	5.5	Nd

where: TRYPT-tryptamine, FEN-phenylethylamine, PUT-putrescine, CAD-cadaverine, HIST-histamine, SER-serotonin, TYR-tyramine, SPD-spermidine, and SPM-spermine. Nd-not detected.

The values presented in table 1 are an average of three determinations for each sample.

Tryptamine and spermine had some samples with 0mg/kg while the rest of samples had concentration below the detection limit. Also, below the detection limit we found in descending number cadaverine, histamine putrescin and

tyramine content in frozen mackerel samples.

It is important to highlight that for tyramine we have the highest quantities of all the biogenic amines. Tyramine maximum value of all the samples analyzed was 42.6mg/kg. It is possible that high values of tyramine to be in the fishes that were unfrozen during transportation or manipulation, which means that mackerel

cold chain, was interrupted. Another possible cause might be the fact that the fish was caught in hot weather (in summer) and was not frozen as quickly as possible. Another possibility was that the ungutted mackerel before freezing were hurt and bacteria from intestine to action at fish meat. Tyramine is a naturally occurring amino acid that forms from the breakdown of protein in food as it ages. Though tyramine helps regulate blood pressure, it can also affect the human body in many different, negative ways. It has been known to trigger headaches and severe migraines. In our case tyramine do not exceed 50 mg/kg and theoretically do not threaten the human health because is in low amount. Strong [7] has shown that 1 mg tyramine can trigger an attack in one particular individual while provocation tests made by Hanington et al.[8] used up to 100mg tyramine. So, we cannot say that peoples that eat the fishes that contain tyramine will get sick and have headaches or high blood pressure. The symptoms vary from individual to individual, because some of them tolerate this biogenic amine better than other.

Frozen mackerel samples had histamine content between Nd to 2.6mg/kg. We can say that 2.6mg/kg is not a high value that threatens the human health. Histamine is a toxic agent implicated in human scombroid poisoning. In hystamine intoxication the symptoms are at the beginning the following: facial flushing/sweating,

dizziness, nausea, and headache, tachycardia, followed by rush, diarrhoea and abdominal cramps. Jarisch and Wantke who used 50 mg/kg of histamine for predisposed individuals observed all these symptoms. [9]. Also, the Commission Regulation (EC) No 2073/2005 (OJ L338, p1, 22/12/2005) of 15 November 2005 on microbiological criteria for foodstuffs specify that for fishery products from fish species associated with a high amount of histidine, the histamine content should be between 100 and 200 mg/kg. In frozen mackerel studied samples the inferior limit is wide off

Putrescin give us an interesting view. We know that cadaverine and putrescin appear in altered fishes. The presence of those biogenic amines, even in small quantities (cadaverine maximum quantity is 1.3 mg/kg and putrescin maximum quantity is 9.6 mg/kg) without exceeding 10 mg/kg, shows us that fishes were in incipient state of spoilage. It is well known that fishes that are caught in warm weather and with guts inside spoil faster.

Spermidine content of mackerel varies from 0 to 5.5 mg/kg.

Of our samples, it appears that sample 6 have five biogenic amines and characterizes the spoiled sample.

Smoked mackerel samples do not contain serotonin at all. Spermine is not present because it has many values of Nd and some of 0 mg/kg.

Table 2.

Biogenic amines content of smoked mackerel samples

Sample number	Biogenic amines (mg/kg)								
	TRYPT	FEN	PUT	CAD	HIST	SER	TYR	SPD	SPM
1	12.9	Nd	Nd	Nd	Nd	Nd	0	1	0
2	20	22	43.8	37.5	35.9	Nd	19	5.8	Nd
3	7.8	Nd	2.5	Nd	Nd	Nd	8.5	4.6	Nd
4	Nd	Nd	16	Nd	Nd	Nd	4.1	7.2	Nd
5	Nd	Nd	13.3	7.4	1.2	Nd	2.4	3.4	Nd
6	0	Nd	Nd	5.8	11.6	Nd	1.9	3.2	Nd
7	3	Nd	5.6	1.1	Nd	Nd	4.7	2.9	Nd
8	0	Nd	4	Nd	6.8	Nd	Nd	3.9	Nd
9	2.2	Nd	7.8	2.6	14.9	Nd	8.6	6	0
10	1.4	Nd	1.7	15.4	7.9	Nd	5.3	2.8	0

Tryptamine is present in eight of the ten samples analyzed. Tryptamine values for smoked mackerel samples are between Nd to 20 mg/kg. On human body, tryptamine has a similar effect like vasoactive amine tyramine. Tyramine is the carboxylated form of tryptophan. This is due to the microorganism action on amino acid tryptophan, and so, it is a product of certain spoilage microorganisms. Very interesting is the presence of phenylethylamine in sample number two. Also, it is in large amount. Phenylethylamine is created by foods breaking down or spoiling.

Putrescin and cadaverine are found in many samples. Also are histamine and tyramine. Their presence clearly indicates the mackerel spoilage. Because for the most of samples the values are relatively small, we can say that the mackerel was already before the smoking treatment in incipient spoilage state. Sample number two is especially the most spoiled of all analyzed samples for smoked mackerel.

Making a comparison between frozen and smoked mackerel, we can say that smoked mackerel has higher content of biogenic amines. This is probably due to the fact that the mackerel was imported in frozen state, it had to be thawed and possibly ungutted before smoking, and those operations influenced the microbiota activity in mackerel leading to biogenic amines formation in higher quantities than in the frozen mackerel.

Conclusion

The Romanian studies on mackerel samples showed clearly that smoked mackerel had more biogenic amines than frozen mackerel.

The amount of biogenic amines found in frozen samples showed that some of the mackerel samples were in incipient state of spoilage. We have also have found that the majority of smoked mackerel was in

incipient state of spoilage. The smoked mackerel samples had higher content of biogenic amines comparing with frozen mackerel ones.

Histamine and tyramine content for frozen and smoked samples do not cause health problems, maybe with one exception, but for healthy individuals they surely do not cause any health problems.

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