



THE EVOLUTION OF SOME BIOCHEMICAL INDICES DURING CHILLING OF FOUR FRESHWATER FISH SPECIES

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Abstract: *The purpose of this paper was to study the evolution of some biochemical parameters in freshwater fish, subjected to chilling for 72 hours in fresh and after smoking. The biological material was represented by four species of fish (catfish, trout, carp and grayling) from which there were prepared fresh and hot smoked (60 minutes at +70°C) samples, then chilled at +5°C for 72 hours. After 12, 48 and 72 hours of refrigeration the following aspects were determined: Acidity Index (AI) and Peroxide Index (PI) of the fat, and Amine Nitrogen (AN). Both after 12 and after 72 hours of chilling, the largest percentage increases of biochemical indices, as compared to controls (fresh and smoked samples before refrigeration), were recorded in unsmoked samples. During the 72 hours of refrigeration, in all analyzed cases, the AN has recorded the highest growth, followed, in order, by AI and PI. The evolution of PI values during chilling of the four freshwater fish species, have shown the freshness maintenance of fat just in catfish and carp, both in fresh samples and in smoked ones. Regarding AN of the four analyzed fish species, the values obtained have indicated a relative freshness of meat after 72 hours (catfish and carp) or even after 48 hours of refrigeration (trout and grayling).*

Keywords: *fish, chilling, smoking, acidity index, peroxide index, amine nitrogen.*

1. Introduction

The chilling and freezing are conservation methods using low temperatures, which seeks to prevent or limit the modification of nutritional and sensory qualities of raw materials and foodstuffs.

Regarding fishes, the deterioration of quality of both wild and farmed fish species is mainly due to action of intrinsic enzymes and microbes [1,2,3]. According to Pigott and Tucker (1990), in order to extend the keeping quality of fish it should lower their body temperature. The freeze-thaw accelerated protein and lipid oxidation, changed the structure of the

myofibrillar protein, caused muscle discoloration, and led to the loss of myofibrillar protein function [5,6]. According to Kong et al. (2013), due to their high levels of long-chain polyunsaturated fatty acids, the fish products are very susceptible to oxidation leading to the formation of lipid hydroperoxides and free radicals.

By Arvanitoyannis and Kotsanopoulos (2012), the smoking process has been used for hundreds of years either to preserve and extend the shelf-life of foods or to give them specific organoleptic characters (taste, flavour).

In recent decades, smoking was widely applied because of the inactivation effect

of smoke (and thermal processing) on harmful enzymatic compounds and microorganisms [9].

In this paper it has investigated the evolution of biochemical indices (acidity and peroxide indices of fats and amino nitrogen) in four species of freshwater fish, subjected to chilling for 72 hours in fresh and after smoking.

2. Experimental

The biological material was represented by four species of fish caught in streams in Romania: catfish (*Silurus glanis* L.), trout (*Salmo trutta* L.), carp (*Cyprinus carpio* L.) and grayling (*Thymallus thymallus* L.) with weights ranging from 0.600 kg (trout) and 1.6 kg (catfish). Fishes were brought fresh (in containers of water) at laboratory for analysis, where they were slaughtered, and after evisceration fresh samples were prepared, which were refrigerated (at +5°C) for 72 hours. In parallel, from the same biological material were prepared samples subjected to hot smoking process (60 minutes at +70°C), and then were chilled under the same conditions as the fresh ones (not smoked). All samples were placed in individual sealed plastic bags and then refrigerated. From fish fat (extracted by pressing) were evaluated Peroxide Index (PI) and Acidity Index (AI), and from meat Amino Nitrogen (AN). Fresh and smoked samples were analyzed both before and during the chilling, after 12, 48 and 72 hours.

The pH values were determined with a digital pH-meter type Hanna.

The Peroxide Index - PI (g iodine/100g fat) was determined by titration with

Na₂S₂O₃ 0.01 N solution of iodine released from KI by peroxides formed in oxidized fat [10,11]. The assessment of the degree of impaired fat oxidation is as follows: PI<0.03 is considered fresh fat; IP=0.03 to 0.06 fat is fresh, but not recommended for storage; IP=0.06 to 0.1 fat is questionable freshness, and the IP>0.1 fat is altered.

The determination of Acidity Index - AI (mg KOH/g) was made through a titration method, based on measurement of volume of KOH 0,1 N solution, which neutralizes free fatty acids from one gram of fat [10].

The amine (trimethylamine) nitrogen - AN (mg%) was determined by the difference between the nitrogen content of volatile bases and the nitrogen content of the ammonia, and primary amines [10]. After Castell and Triggs (cited by Beschea and Toma [10]), the admissibility limits of fish depending on the content of trimethylamine are: 0-1 mg/100 g (fresh fish); 1-5 mg/100 g of product (relative freshness of fish); above 5 mg/100 g (altered fish).

Four replicates for each determination represented the data of experiments, which were statistically processed, the analysis of variance being used to calculate differences between results. The differences at p < 0.05 were considered significant.

3. Results and discussion

In the Table 1 are reproduced the values of pH, Acidity Index (AI), Peroxide Index (PI) and Amine Nitrogen (AN) from fresh and smoked samples, belonging to: catfish, trout, carp and grayling.

Table 1

The mean values of biochemical indices of fish species before and after smoking

Fish species	Fish status	pH	AI	PI	AN
Catfish	Unsmoked*	6.45	2.03±0.04	0.019±0.004	0.22±0.002
	Smoked	6.62	3.74±0.01	0.022±0.006	0.24±0.005
Trout	Unsmoked*	6.35	3.70±0.09	0.024±0.004	0.42±0.003
	Smoked	6.53	6.25±0.07	0.032±0.001	0.48±0.008
Carp	Unsmoked*	6.31	3.57±0.08	0.021±0.002	0.25±0.007
	Smoked	6.54	6.08±0.03	0.028±0.005	0.36±0.001
Grayling	Unsmoked*	6.27	4.35±0.05	0.025±0.001	0.31±0.001
	Smoked	6.50	6.39±0.01	0.037±0.003	0.38±0.003

* Fresh; AI=Acidity Index; PI=Peroxide Index; AN=Amine Nitrogen

From Table 1 it is observed that, in all four analyzed species, as result of smoking process, pH values, AI, PI and AN have registered more or less pronounced increases compared to fresh samples.

The Table 2 restores the values of AI, PI and AN in samples of fresh and smoked fish, which have been kept refrigerated for 72 hours.

Table 2

The mean values of biochemical indices during chilling of the four fish species

Fish species	Status of	Cooling	AI	PI	AN
Catfish	Unsmoked	12	2.35±0.01	0.019±0.002	0.50±0.006
		48	4.94±0.04	0.027±0.003	0.62±0.003
		72	7.40±0.09	0.043±0.001	1.27±0.001
	Smoked	12	4.25±0.07	0.025±0.003	0.38±0.002
		48	5.30±0.08	0.032±0.006	0.42±0.008
		72	6.08±0.02	0.048±0.005	0.81±0.005
Trout	Unsmoked	12	5.35±0.01	0.031±0.001	0.88±0.001
		48	9.90±0.07	0.043±0.002	1.72±0.003
		72	16.64±0.72	0.064±0.007	3.04±0.007
	Smoked	12	8.82±0.06	0.039±0.003	0.62±0.001
		48	10.36±0.04	0.052±0.008	1.14±0.003
		72	14.23±0.03	0.073±0.004	1.56±0.009
Carp	Unsmoked	12	4.12±0.04	0.028±0.007	0.63±0.005
		48	8.23±0.07	0.027±0.002	0.93±0.002
		72	14.68±0.05	0.058±0.005	1.85±0.006
	Smoked	12	7.15±0.02	0.034±0.009	0.52±0.007
		48	10.53±0.07	0.037±0.001	0.78±0.003
		72	13.90±0.01	0.056±0.002	1.03±0.002
Grayling	Unsmoked	12	6.02±0.04	0.033±0.008	0.75±0.009
		48	8.45±0.02	0.045±0.003	1.35±0.004
		72	12.38±0.03	0.061±0.006	2.43±0.005
	Smoked	12	7.45±0.08	0.043±0.005	0.69±0.003
		48	9.67±0.02	0.049±0.001	1.02±0.002
		72	11.74±0.05	0.062±0.002	1.23±0.009

AI=Acidity Index; PI=Peroxide Index; AN=Amine Nitrogen

As seen from the Table 2, in unsmoked samples of catfish AI has increased, during chilling, with percentages between 15.7% (after 12 hours) and 254.5% (after 72 hours), as compared to fresh sample before chilling (Tab. 1).

Also at chilled catfish, but in smoked samples, AI has increased by 13.6% (after 12 hours) and by 62.6% (after 72 hours), compared to smoked samples before chilling (Tab. 1) ($p < 0.05$).

In unsmoked catfish samples PI has increased during chilling with percentages between 0% (after 12 hours) and 126.3% (after 72 hours), compared to fresh samples prior to chilling.

In smoked catfish sample IP increased, during chilling, by 13.6% (after 12 hours) and by 118.2% (after 72 hours), as compared to smoked sample before chilling ($p < 0.05$).

PI values of catfish shows that, during chilling, the fat of this fish maintained this state of freshness, both in smoked and fresh samples.

Also in catfish (Tab. 2), the amine nitrogen values (AN) in unsmoked samples have increased, during the refrigeration, with percentages between 127.3% (after 12 hours) and 577.3% (after 72 hours) as compared to fresh samples before chilling.

In smoked and chilled samples, AN has increased by 58.3% (after 12 hours) and by 237.5% (after 72 hours), as compared to smoked samples before chilling ($p < 0.05$).

After 72 hours of refrigeration, AN values have indicated in fresh samples (unsmoked) a fish (catfish) with relative freshness.

In unsmoked trout samples (Tab. 2), AI has increased, during chilling, with percentages between 44.6% (after 12 hours) and 349.7% (after 72 hours), as compared to fresh sample before chilling (Tab. 1).

Also in chilled trout, but in smoked samples, AI has increased by 41.1% (after 12 hours) and by 127.7% (after 72 hours), compared to smoked samples before chilling (Tab. 1) ($p < 0.05$).

In unsmoked trout samples PI has increased, during chilling, with percentages between 29.2% (after 12 hours) and 166.7% (after 72 hours), compared to fresh samples prior to chilling (Tab. 1).

In smoked trout samples PI has increased, during chilling, by 21.9% (after 12 hours) and by 128.1% (after 72 hours), as compared to smoked sample before chilling ($p < 0.05$).

PI values of trout shows that, after 72 hours of chilling, both fresh samples but especially the smoked ones have shown a dubious freshness fat.

Also in trout, AN values in unsmoked samples have increased, during refrigeration, with percentages between 109.5% (after 12 hours) and 623.8% (after 72 hours), as compared to fresh samples before chilling.

In smoked and chilled trout samples, AN has increased by 29.2% (after 12 hours) and 225% (after 72 hours) compared to smoked samples before chilling ($p < 0.05$).

The extension of refrigeration state over 48 hours has led to higher AN values, both in fresh and in smoked samples, indicating, in both cases, a fish with relative freshness.

In unsmoked carp samples (Tab. 2), AI has increased, during chilling, with percentages between 15.4% (after 12 hours) and 311.2% (after 72 hours), as compared to fresh sample before chilling (Tab. 1).

Also in chilled carp, but in smoked samples, AI has increased by 17.6% (after 12 hours) and by 128.6% (after 72 hours), compared to smoked samples before chilling (Tab. 1) ($p < 0.05$).

In unsmoked carp samples PI has increased, during chilling, with percentages between 33.3% (after 12 hours) and 166.7% (after 72 hours), compared to fresh samples prior to chilling (Tab. 1).

In smoked carp samples PI has increased, during chilling, by 21.4% (after 12 hours) and by 100% (after 72 hours), as compared to smoked sample before chilling ($p < 0.05$).

Analyzing PI values in carp samples, it is observed that throughout the chilling the fat of this fish has maintained its freshness status, both in fresh and smoked samples

Also in carp, AN values in unsmoked samples have increased, during refrigeration, with percentages between 152% (after 12 hours) and 640% (after 72 hours), as compared to fresh samples before chilling (Tab. 1).

In smoked and chilled carp samples, AN has increased by 44.4% (after 12 hours) and by 186.1% (after 72 hours), compared to smoked samples before chilling ($p < 0.05$).

After 72 hours of refrigeration, AN values have indicated in fresh samples (unsmoked) a fish with relative freshness.

In unsmoked grayling samples (Tab. 2), AI has increased, during chilling, with percentages between 38.4% (after 12 hours) and 184.6% (after 72 hours), as compared to fresh sample before chilling (Tab. 1).

Also in chilled grayling, but in smoked samples, AI has increased by 16.6% (after 12 hours) and by 83.7% (after 72 hours), compared to smoked samples before chilling (Tab. 1) ($p < 0.05$).

In unsmoked grayling samples PI has increased, during chilling, with percentages between 32% (after 12 hours) and 144% (after 72 hours), compared to fresh samples prior to chilling (Tab. 1).

In smoked grayling samples PI has increased, during chilling, by 16.2% (after

12 hours) and by 67.6% (after 72 hours), as compared to smoked sample before chilling ($p < 0.05$).

After 72 hours of refrigeration, PI values have indicated, both in fresh and smoked samples, a fat with dubious freshness.

Also in grayling, AN values in unsmoked samples have increased, during refrigeration, with percentages between 141.9% (after 12 hours) and 683.9% (after 72 hours), as compared to fresh samples before chilling (Tab. 1).

In smoked and chilled grayling samples, AN has increased by 100% (after 12 hours) and by 223.7% (after 72 hours), compared to smoked samples before chilling ($p < 0.05$).

48 hours of refrigeration, in fresh samples (not smoked), and 72 hours, in smoked samples, have made that AN values indicate, in both cases, a fish (grayling) with relative freshness.

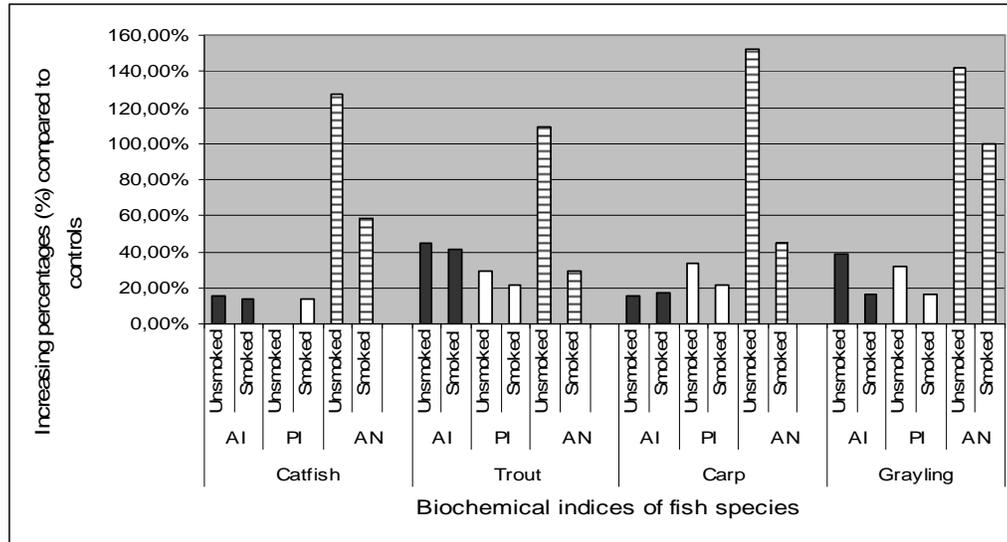
According to Horner (1997), besides surface drying and salting process, the food protection is provided by antioxidant compounds and several antimicrobial agents like phenols, nitrites etc. Phenolic compounds generated by the combustion combined with the temperature and the conditions of smoking can reduce the microbiological development and the oxidation [13].

Kyriazi-Papadopoulou et al. (2003) evaluated the qualitative and safety characteristics of Mediterranean mussel (*Mytilus galloprovincialis*) during storage at 2–3°C, under vacuum packaging, after washing and steaming (at 80°C, for 10 min), salting (brine 4%, 15 min), draining (60–65°C for 13 min), and smoking (at 65–80°C for 17 min). Under these conditions, the product could be preserved for up to 70 days; the authors found a dropping of pH, an increase of thiobarbituric acid, and a marked increase of total volatile basic nitrogen (TVB-N) values.

Searching the effects of hot smoking in combination with marinating and vacuum packaging, on the qualitative characteristics of mackerel (*Scomber scombrus*) stored at 4°C, Ozogul and Balikci (2011) found several fluctuations of total volatile basic nitrogen (TVB-N) level, throughout storage period, while the

level of fatty acids enhanced. Smoking in combination with marinating and vacuum packaging preserved the fish quality, the shelf-life of products was 9 months.

Fig. 1 illustrates, comparatively, the percentage increases of the three biochemical indices, after 12 hours of refrigeration.



AI=Acidity Index (mg KOH/g); PI=Peroxide Index (g iodine/100 g fat), AN=Amine Nitrogen (mg%)

Fig. 1. Percentage increases of biochemical indices in fish species after 12 hours of refrigeration, compared to controls (fresh samples)

From the figure it is observed that, after 12 hours of fish chilling, the largest percentage increases of biochemical indices, compared to controls (fresh and smoked samples before chilling), were recorded in unsmoked samples.

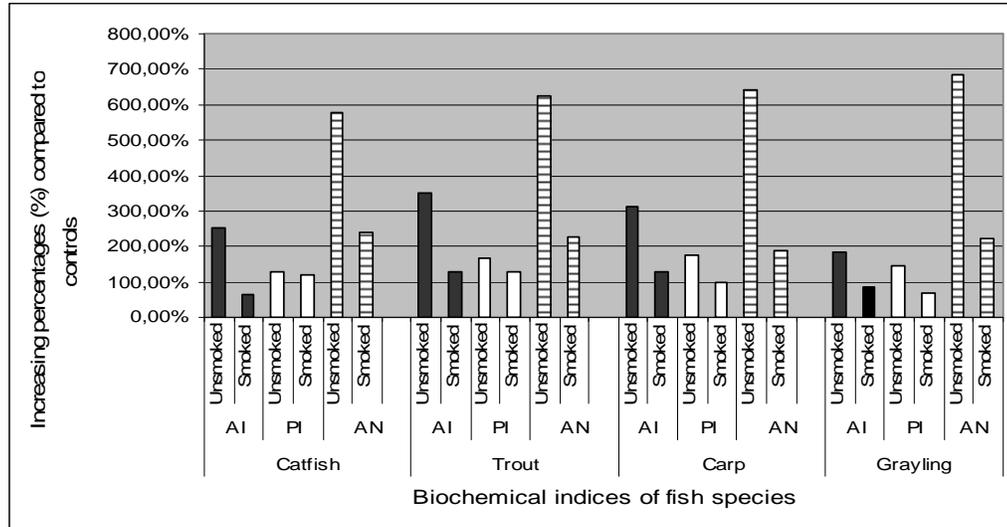
In unsmoked samples, one can see that AN has recorded the largest increases in carp (152%) and the lowest in trout (109.5%), AI in trout (44.6%) and the lowest in catfish (15.7%) and carp (15.4%), and PI the biggest in carp (33.3%) and the lowest in catfish (0%).

In smoked samples, AN has recorded the largest increases in grayling (100%) and

the lowest in trout (29.2%), AI in trout (41.1%) and the lowest in catfish (13.6%), and PI the higher in trout (21.9%) and lowest in catfish (13.6%).

Fig. 2 reproduces, comparatively, the percentage increases of the biochemical indices, after 72 hours of refrigeration.

After 72 hours of refrigeration of the four fish species, the largest percentage increases of the analyzed biochemical indices, compared to controls (fresh and smoked samples before chilling), were recorded in unsmoked samples too.



AI=Acidity Index (mg KOH/g); PI=Peroxide Index (g iodine/100 g fat), AN=Amine Nitrogen (mg%)
Fig. 2. Percentage increases of biochemical indices in fish species after 72 hours of refrigeration, compared to controls (fresh samples)

In unsmoked samples, AN has recorded the largest increases in grayling (683.9%) and the lowest in catfish (577.3%), AI in trout (349.7%) and the lowest in grayling (184.6%), and PI the biggest in carp (176.2%) and lowest in catfish (126.3%).

In smoked samples, AN has recorded the largest increases in catfish (237.5%) and the lowest in carp (186.1%), AI in carp (128.6%) and trout (127.7%), and the lowest in catfish (62.6%), and PI the higher in trout (128.1%) and lowest in grayling (67.6%).

4. Conclusions

The evolution of the Peroxide Index (PI), Acidity Index (AI) and Amine Nitrogen (AN) during chilling (6°C) of four freshwater fish species (catfish, trout, carp and grayling) has shown differences, both between fresh (not smoked) samples and smoked of the same species, and between different species.

So after 12 and after 72 hours of refrigeration, the largest percentage

increases of biochemical indices, compared to controls (fresh and smoked samples before chilling), were recorded in unsmoked samples

Thus, after 12 hours of refrigeration of unsmoked samples, AN has recorded the highest increases in carp and the lowest in trout; AI in trout and the lowest in catfish and carp, and PI the largest increases in carp and lowest in catfish.

At smoked samples, AN has recorded the most marked increases in grayling and the smallest in trout; AI in trout, and the smallest in catfish, and PI the largest increases in trout and lowest ones in catfish.

After 72 hours of refrigeration of unsmoked samples, AN has recorded the largest increases in grayling and the smallest in catfish; AI in trout, and the lowest in grayling, and PI the largest in carp and the lowest in catfish.

At smoked samples, AN has recorded the highest increases in catfish and the smallest in carp; AI in carp and trout, and the lowest in catfish, and PI the largest increases in trout, and the lowest in grayling.

During the 72 hours of refrigeration, in all analyzed cases, AN has recorded the highest growth, followed, in order, by AI and PI.

The evolution of PI values during chilling of the four freshwater fish species, has shown the freshness maintenance of fat just in catfish and carp, both in fresh samples and in smoked ones.

Regarding AN of the four analyzed fish species, the values obtained have indicated a relative freshness of meat after 72 hours (catfish and carp) or even after 48 hours of refrigeration (trout and grayling).

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