THE EVOLUTION OF TWO BIOCHEMICAL INDICES DURING CHILLING OF SOME FISH SPECIES

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Abstract: The evolution of two biochemical parameters (acidity index of fats and amino nitrogen) in four fish species (two of fresh water, and two of salt water), subjected to chilling for 72 hours (fresh and smoked) was the purpose of this paper. The research materials were represented by four species of fish: catfish, carp, bream and mackerel, from which were prepared fresh and smoked samples, then chilled at +6°C for 72 hours. After 24, 48 and 72 hours of refrigeration the Acidity Index (AI) of the fat, and Amino Nitrogen (AN) were determined. Both after 24 and after 72 hours of chilling, the largest percentage increases of biochemical indices, compared to controls (fresh and smoked samples before refrigeration), were recorded in fresh samples. Compared to samples before chilling, after 72 hours of refrigeration, AI registered the largest increase in bream and the lowest in carp (fresh samples), and the largest increases in carp and the lowest in catfish (smoked samples). AN values indicated a relative freshness of meat after 24 hours of chilling (fresh mackerel), after 48 hours (smoked mackerel, fresh and smoked bream, fresh carp and catfish), after 72 hours (smoked carp), and even altered meat in catfish, after 72 hours of chilling.

INTRODUCTION

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

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) (*Arvanitoyannis and Kotsanopoulos, 2012*).

In recent decades, smoking was widely applied because of the inactivation effect of smoke (and thermal processing) on harmful enzymatic compounds and microorganisms (*Šimko, 2002*).

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 (*Kong et al. (2013*). The deterioration of quality of both wild and farmed fish species is mainly due to action of intrinsic enzymes and microbes (*Pigott and Tucker, 1987; Hsieh and Kinsella, 1989; Mehta Naresh Kumar et al., 2011*).

By Pigott and Tucker (1990), in order to extend the keeping quality of fish it should lower their body temperature, but 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 (*Xia et al., 2009; Xia et al., 2010*)

In this paper it has investigated the evolution of biochemical indices (acidity index of fats and amino nitrogen) in four fish species (two of fresh water, and two of salt water), subjected to chilling for 72 hours (fresh and smoked), to see how influence the fish status and the storage duration the value of indices analyzed and the meat freshness.

MATERIALS AND METHODS

Research materials. The materials were represented by four species of fish: catfish (*Silurus glanis* L.), and carp (*Cyprinus carpio* L.), caught in romanian streams, bream (*Sparus aurata* Linnaeus) and mackerel (*Scomber japonicus* Houttuyn) caught in the Black Sea.

The preparation of samples. The fishes, whose weight ranged from 0.450 kg (bream) to 1.2 kg (catfish), were brought fresh (in containers of water) at laboratory, where they were slaughtered, and after evisceration fresh samples were prepared. In parallel, from the same biological material were prepared samples subjected to hot smoking process (60 minutes at +70°C). All samples were placed in individual sealed plastic bags and then refrigerated at + 6°C for 72 hours.

From fish fat (extracted by pressing) was evaluated Acidity Index (AI), and from meat Amino Nitrogen (AN). Fresh and smoked samples were analyzed both before and during the chilling, after 24, 48 and 72 hours.

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

The 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 amino (trimethylamine) nitrogen - AN (mg%) was evaluated 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, 1984), 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).

Statistical analysis. The data of experiments, consisting in four replicates for each determination, were statistically processed, and the analysis of variance was used to calculate differences between results. The differences at $p<0.05$ were considered significant.

RESULTS AND DISCUSSION

In the Table 1 are reproduced the comparative values of pH, Acidity Index (AI) and Amino Nitrogen (AN) in four fish species before chilling.

Table 1.The mean values (±SD) of fishes biochemical indices before chilling

Fish species	Fish status	pH	AI (mg KOH/g)	AN (mg%)
Catfish	Fresh*	6.38	2.12±0.04	0.48±0.003
	Smoked	6.57	3.55±0.02	0.52±0.008
Carp	Fresh	6.34	3.49±0.06	0.39±0.005
	Smoked	6.56	5.18±0.03	0.43±0.004
Bream	Fresh	6.41	3.15±0.07	0.50±0.005
	Smoked	6.58	5.23±0.05	0.57±0.009
Mackerel	Fresh	6.37	4.02±0.06	0.76±0.004
	Smoked	6.52	5.79±0.03	0.82±0.007

SD=standard deviation;*=Unsmoked; AI=Acidity Index; AN=Amino Nitrogen

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

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

Table 2. The mean values (±SD) of biochemical indices during chilling of fish species

Fish species	Chilled fish status	Cooling duration	AI (mg KOH/g)	AN (mg%)
Catfish	Fresh*	24 hours	3.43±0.04	0.74±0.005
		48 hours	5.07±0.03	1.65±0.004
		72 hours	7.35±0.08	5.02±0.003
	Smoked	24 hours	4.19±0.06	0.67±0.003
		48 hours	5.26±0.04	1.17±0.003
		72 hours	6.18±0.05	2.35±0,007
	Fresh	24 hours	4.23±0.07	0.64±0.004
		48 hours	5.89±0.05	1.27±0.007

Carp		72 hours	8.68±0.05	2.85±0.006
	Smoked	24 hours	6.15±0.07	0.52±0.004
		48 hours	10.48±0.05	0.98±0.008
		72 hours	11.25±0.08	1.73±0.01
Bream	Fresh	24 hours	4.07±0.05	0.74±0.007
		48 hours	5.53±0.07	1.32±0.009
		72 hours	11.64±0.72	2.54±0.02
	Smoked	24 hours	5.81±0.04	0.72±0.005
		48 hours	7.26±0.06	1.04±0.009
		72 hours	9.45±0.08	1.56±0.009
Mackerel	Fresh	24 hours	5.17±0.07	1.07±0.009
		48 hours	6.8±0.09	1.65±0.007
		72 hours	10.6±0.08	2.93±0.009
	Smoked	24 hours	6.52±0.04	0.94±0.005
		48 hours	8.09±0.09	1.47±0.008
		72 hours	11.93±0.1	2.05±0.006

SD=standard deviation;*=Unsmoked; AI=Acidity Index; AN=Amino Nitrogen

In the Table 2 one can see that in fresh (unsmoked) samples of catfish AI increased, during chilling, with percentages ranged from 61.8% (after 24 hours) to 246.7% (after 72 hours), as compared to fresh sample before chilling (Tab. 1).

In chilled samples of smoked catfish, AI increased by 18.03% (after 24 hours) and by 74.1% (after 72 hours), compared to smoked samples before chilling (Tab. 1) ($p<0.05$).

In catfish, the amino nitrogen values (AN) in fresh samples have increased, during the refrigeration, with percentages between 54.2% (after 24 hours) and 945.8% (after 72 hours) as compared to fresh samples before chilling (Tab. 2).

In smoked catfish during chilling, AN has increased by 28.8% (after 24 hours) and by 351.9% (after 72 hours), as compared to smoked samples before chilling ($p<0.05$).

After 48 hours of refrigeration of catfish, AN values have indicated in fresh samples (unsmoked) a relative freshness, and after 72 hours an altered fish.

In fresh carp samples (Tab. 2), AI has increased, during chilling, with percentages between 21.2% (after 24 hours) and 148.7% (after 72 hours), as compared to fresh sample before chilling (Tab. 1).

Also in chilled carp, but in smoked one, AI has increased by 18.7% (after 24 hours) and by 155.8% (after 72 hours), compared to smoked samples before chilling (Tab. 1) ($p<0.05$).

In carp, AN values in fresh samples have increased, during refrigeration, with percentages between 64.1% (after 24 hours) and 630.8% (after 72 hours), as compared to fresh samples before chilling.

In smoked carp during chilling, AN has increased by 20.9% (after 24 hours) and 302.3% (after 72 hours) compared to smoked samples before chilling ($p<0.05$).

The extension of refrigeration over 48 hours (in fresh samples), and over 72 hours (in smoked samples) has led to increased values of AN, indicating, in both cases, carp samples with relative freshness.

In fresh bream samples (Tab. 2), AI has increased, during chilling, with percentages between 26.03% (after 24 hours) and 269.5% (after 72 hours), as compared to fresh sample before chilling (Tab. 1).

In chilled bream, but in smoked samples, AI has increased by 11.1% (after 24 hours) and by 80.7% (after 72 hours), compared to smoked samples before chilling ($p<0.05$).

During refrigeration of bream, AN values in fresh samples have increased with percentages between 48% (after 24 hours) and 408% (after 72 hours), as compared to fresh samples before chilling (Tab. 1).

In smoked bream during chilling, AN has increased by 26.3% (after 24 hours) and by 173.7% (after 72 hours), compared to smoked samples before chilling ($p<0.05$).

After 48 hours of bream refrigeration, AN values have indicated both in fresh and in smoked samples a relative freshness.

In fresh mackerel samples (Tab. 2), AI has increased, during chilling, with percentages between 28.6% (after 24 hours) and 153.7% (after 72 hours), as compared to fresh sample before chilling (Tab. 1).

In smoked samples of mackerel, AI has increased by 12.6% (after 24 hours) and by 106% (after 72 hours), compared to smoked samples before chilling ($p<0.05$).

In fresh samples of mackerel, AN values have increased, during refrigeration, with percentages between 40.8% (after 24 hours) and 285.5% (after 72 hours), as compared to fresh samples before chilling.

In smoked mackerel during chilling, AN has increased by 14.6% (after 24 hours) and by 150% (after 72 hours), compared to smoked samples before chilling ($p<0.05$).

24 hours of chilling, in fresh (not smoked) mackerel, and 48 hours, in smoked mackerel, have made that AN values to indicate, in both cases, samples with relative freshness.

Besides surface drying and salting process, the food protection is provided by antioxidant compounds and several antimicrobial agents like phenols, nitrites etc. (Horner, 1997). According to Varlet et al. (2007), phenolic compounds generated by the combustion combined with the temperature and the conditions of smoking can reduce the microbiological development and the oxidation.

Studying 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.), Kyriazi-Papadopoulou et al. (2003) saw that the product could be preserved for up to 70 days. But the same authors found a dropping of pH (by 0.2), an increase of thiobarbituric acid, and a marked increase of total volatile basic nitrogen (TVB-N) values.

Ozogul and Balikci (2011) searched the effects of hot smoking in combination with marinating and vacuum packaging, on the qualitative characteristics of mackerel (*Scomber scombrus*) stored at 4°C. Under these conditions, the two authors found several fluctuations of total volatile basic nitrogen (TVB-N) level, throughout storage period, while the level of fatty acids enhanced (from 2.46 to 7.43). 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 biochemical indices, after 24 hours of refrigeration.

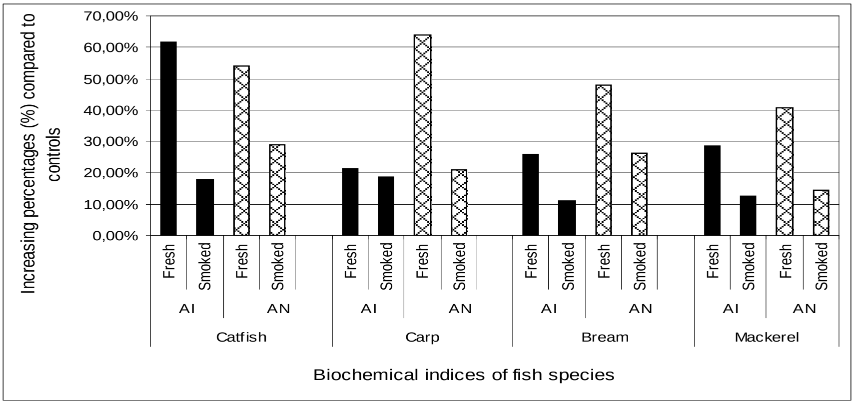

Fig. 1. Percentage increases of biochemical indices in fish species after 24 hours of refrigeration, compared to controls (samples before chilling)
AI=Acidity Index (mg KOH/g); AN=Amino Nitrogen (mg%)

From the figure it is observed that, after 24 hours of fish chilling, the largest percentage increases of biochemical indices, compared to controls (fresh and smoked samples before chilling), were recorded in fresh (not smoked) samples.

In fresh samples, one can see that AI has recorded the largest increase in catfish (61.8%) and the lowest in carp (21.2%), while AN had the largest increase in carp (64,10%) and the lowest in mackerel (40.8%).

In smoked samples, AI has recorded the largest increase in carp and catfish (~18%) and lowest in bream (11,1%). AN has had the largest increase in catfish (28.8%) and the lowest in mackerel (14.6%).

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

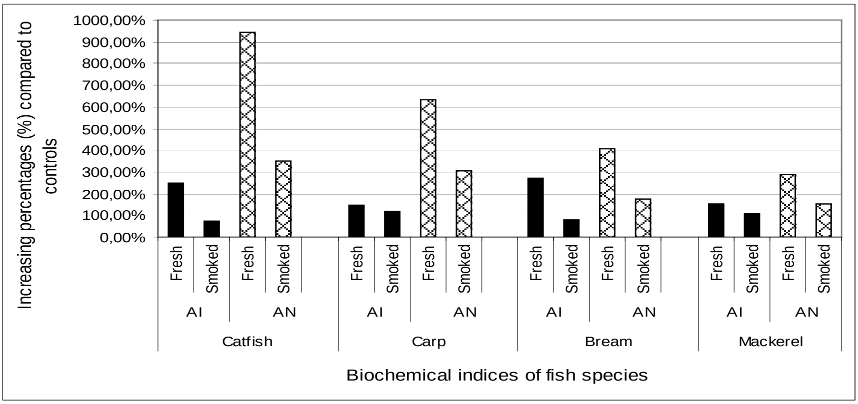

Fig. 2. Percentage increases of biochemical indices in fish species after 72 hours of refrigeration, compared to controls (samples before chilling)

AI=Acidity Index (mg KOH/g); AN=Amino Nitrogen (mg%)

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

In fresh samples, AI has recorded the largest increase in bream (269.5%) and the lowest in carp (148.7%), while AN had the largest increase in catfish (945.8%) and the lowest in mackerel (285.5%).

In smoked samples, AI has registered the largest increases in carp (117.2) and the lowest in catfish (74.1%). AN has had the largest increase in catfish (351.9%) and the lowest in mackerel (150%).

CONCLUSIONS

The evolution of the Acidity Index (AI) and Amino Nitrogen (AN) during chilling (6°C) of four fish species (catfish, carp, bream and mackerel) has shown differences, both between fish status (fresh and smoked), and between species.

After 24 and 72 hours of refrigeration, the largest percentage increases of biochemical indices, compared to controls (fresh and smoked samples before chilling), were recorded in fresh (not smoked) samples.

Compared to samples before chilling, after 24 hours of refrigeration AI registered the largest increase in catfish and the lowest in carp (fresh samples), and the largest increase in carp and catfish (close values), and the lowest in bream (smoked samples).

Compared to samples before chilling, after 72 hours of refrigeration, AI registered the largest increase in bream and the lowest in carp (fresh samples), and the largest increases in carp and the lowest in catfish (smoked samples).

The analyse of AN values indicated a relative freshness of meat after 24 hours of chilling (fresh mackerel), after 48 hours (smoked mackerel, fresh and smoked bream, fresh carp and catfish), after 72 hours (smoked carp), and even altered meat in catfish, after 72 hours of chilling.

During the 72 hours of chilling, in all cases, AN recorded the highest increses compared to AI..

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