Journal homepage: www.fia.usv.ro/fiajournal
Journal of Faculty of Food Engineering,
Ştefan cel Mare University of Suceava, Romania
Volume XVI, Issue 2 - 2017, pag. 92 - 97

THE INFLUENCE OF SLOW THAWING ON EVOLUTION OF SOME BIOCHEMICAL COMPOUNDS IN FROZEN FISHES

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Received May 17rd 2017, accepted 24th June 2017

Abstract: *The aim of this work was to study the evolution of pH, amino nitrogen and nitrogen from aminoacids in four fish species, during 48 hours of slow thawing, in order to assess the fish spoilage speed in these keeping conditions. The biological material was represented by frozen fishes (carp, catfish, mackerel and hake) which were subjected to slow thawing at room temperature (+20..+22°C), by analysing, at certain time intervals, pH, amino nitrogen - AN (mg %), and nitrogen from aminoacids - NAA (g %). The pH was determined with a digital pH-meter type Hanna, and the nitrogen from aminoacids according to Sörensen method. The amino nitrogen was determined by the difference between the nitrogen content of volatile bases and the nitrogen content of the ammonia and primary amines. As compared to frozen samples, both pH and the amino nitrogen values of all fish samples showed constant and significant increases up to the end of the analyzed period, while the nitrogen from aminoacids only in the first 30 hours of thawing. The amino nitrogen and the nitrogen from amino acids values have indicated the highest spoilage speed in catfish and hake, and the least speed in mackerel.*

Keywords: *pH, freshness, amino nitrogen, nitrogen from aminoacids.*

1. Introduction

The conservation methods using low temperatures, such as freezing, can prevent or limit the modification of nutritional and sensory qualities of raw materials and foodstuffs.
As a consequence of biochemical changes taking place in the proteins and lipid fractions during chilling storage of fishes, the deterioration in sensory quality, loss of nutritional value and changes in physico-chemical properties occur [1, 2, 3].
According to Matsumoto [4], some deterioration occurs, including changes in flavour, colour, odour, and texture, during the freezing, thawing, and frozen storage of fish muscle. The freeze-thaw process caused fibre distortion and an increased gap between fibres in whole tiger shrimp [5]. Also, 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 [6, 7].
By some authors [8, 9, 10], the rate of fish outage varies from one species to another; the deterioration of quality of both wild and farmed fish species is mainly due to action of intrinsic enzymes and microbes.
The protein and lipid oxidation occur and have an important influence on product acceptability, during the frozen storage of meat [11]. The fish products are very susceptible to oxidation due to their high levels of long-chain polyunsaturated fatty acids, and this oxidation leads to the formation of lipid hydroperoxides and free radicals [12].

However, there are many compounds, including certain low-molecular-weight sugars and polyols, as well as many amino acids, carboxylic acids and polyphosphates that have cryoprotective properties [13].
In this work, the evolution of pH and two nitrogen compounds was investigated, during slow thawing (+20..+22°C) of four frozen fish species, to see what species have a higher spoilage speed on these conditions.

2. Experimental

2.1. Research material and samples preparation

The biological material was represented by four fish species: carp (*Cyprinus carpio* L.), catfish (*Silurus glanis* L.), mackerel (*Scomber japonicus* Houttuyn), and hake (*Merluccius merluccius* L.), whose weight ranged from 0.250 kg to 0.900 kg. Carp and catfish were caught in romanian streams and brought fresh (in containers of water) at laboratory, where they were slaughtered. After evisceration, meat samples were immediately frozen (at –29°C), and kept two months up to the experiment. Mackerel and hake were bought eviscerated and frozen from retail providers.

2.2. Procedure and research methods

The fish samples was subjected to slow thawing at room temperature (+20...+22°C), analyzing, at certain intervals, pH values, amino nitrogen, and nitrogen from aminoacids.
The amino 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 [14].
The nitrogen from aminoacids, NAA (g %) was determined titrimetrically, according to Sörensen method [14].
The pH values were determined with a digital pH-meter type Hanna.

2.3. Statistical analysis

Four replicates of each determination have represented the data of experiments, which were statistically processed, using SAS Version 8.02 [15]**.** To analyze the significance of differences among samples, generalized linear model analysis was carried out. For multiple comparisons Duncan's multiple range test was used (P<0.05).

3. Results and discussion

The values of pH, amino nitrogen, AN (mg %), and nitrogen from aminoacids, NAA (g %) in frozen fish samples are shown in the table 1.

Table 1

Biochemical indices values in frozen fish samples

Fish species Biochemical indices	Carp	Catfish	Mackerel	Hake
pH	6.35±0.78cc*	6.40±0.91cc*	6.38±1.04cc	6.41±0.37cc
AN (mg %)	0.37±0.02HI*	0.50±0.09HI*	0.73±0.07HI	0.52±0.03HI
NAA (g %)	0.06±0.008d*	0.07±0.005d*	0.05±0.006d	0.04±0.007d

*Means with the same letters within a row are not statistically diferent (P<0.05)

As can be seen from the table, the values of these indices show small and non significant differences between fish species.

In the Table 2 the biochemical indices determined at certain time intervals of the thawing process are reproduced.

Table 2

Biochemical indices values during fish thawing

Test intervals		3 h	9 h	15 h	21 h	30 h	36 h	42 h	48 h
Spl.	**Test**								
FS1	pH	6.38± 0.25 cc*	6.65± 0.78 bc*	6.83± 1.07 bb	7.05± 0.69 bb	7.35± 0.93 ab	7.40± 1.32 ab	7.58± 0.49 ab	7.66± 0.92 aa
	AN (mg %)	0.56± 0.09 HI*	1.53± 0.11 H*	4.68± 1.05 FG	6.03± 1.57 F	8.53± 0.64 E	9.44± 0.09 E	10.33± 1.19 DE	13.75± 0.72 C
	NAA (g %)	0.08± 0.007 cd*	0.09± 0.005 cd	0.18± 0.03 c*	0.23± 0.05 c	0.31± 0.04 bc	0.25± 0.08 c	0.23± 0.03 c	0.17± 0.01 c
FS2	pH	6.40± 0.97 cc	6.74± 0.63 bc	6.90± 0.48 bb	7.12± 0.88 bb	7.46± 0.59 ab	7.58± 0.45 ab	7.64± 1.23 aa	7.85± 0.38 aa
	AN **(**mg %)	1.03± 0.04 H	2.36± 0.07 GH	7.58± 1.37 EF	9.91± 0.89 DE	12.98± 1.88 CD	13.75± 1.75 C	15.08± 1.08 BC	17.13± 1.92 A
	NAA (g %)	0.1± 0.07 cd	0.15± 0.04 cd	0.21± 0.09 c	0.34± 0.07 bc	0.38± 0.09 bc	0.32± 0.05 bc	0.25± 0.06 c	0.19± 0.09 c
FS3	pH	6.42± 1.04 cc	6.65± 0.39 bc	6.73± 0.65 bc	6.94± 0.32 bb	7.35± 0.98 ab	7.37± 0.21 ab	7.49± 0.55 ab	7.58± 0.63 ab
	AN (mg %)	0.88± 0.09 H	0.98± 0.05 H	3.95± 0.74 G	5.27± 0.56 FG	8.03± 1.08 E	9.14± 1.23 E	9.96±1 .08 DE	11.81± 0.98 D
	NAA (g %)	0.07± 0.007 d	0.10± 0.08 cd	0.17± 0.03 c	0.23± 0.09 c	0.29± 0.07 bc	0.25± 0.09 c	0.21± 0.08 c	0.19± 0.06 c
FS4	pH	6.48± 1.02 cc	6.70± 1.12 bc	6.85± 0.74 bb	7.02± 0.88 bb	7.41± 0.56 ab	7.50± 0.93 ab	7.61± 0.38 aa	7.73± 1.12 aa
	AN (mg %)	0.95± 0.08 H	1.19± 0.04 H	4.87± 1.12 FG	6.53± 1.09 F	9.52± 0.89 DE	10.89± 1.34 DE	11.35± 0.88 D	13.30± 1.56 CD
	NAA (g %)	0.10± 0.09 cd	0.14± 0.07 cd	0.27± 0.05 c	0.36± 0.03 bc	0.42± 0.08 b	0.35± 0.05 bc	0.31± 0.04 bc	0.21± 0.07 c

Spl.= samples; FS1=carp; FS2=catfish; FS3=mackerel; FS4=hake; AN=amino nitrogen; NAA=nitrogen from aminoacids; *Means with different letters within a row are statistically diferent (P<0.05)

As compared to the blank sample (frozen fish - Table 1), at 48 hours of ***carp*** slow thawing, the pH of this fish sample recorded an increase by 1.31 pH units. Significant increases of pH values were registered at 9, 15, 30 and 48 hours of thawing (*P<0.05*).

As seen in the Table 2, at 48 hours of slow thawing, the amino nitrogen, **AN** (mg %), of carp has increased by 37.2 times compared with frozen fish (Table 1), the largest and significant increase being recorded in the range 42-48 hours (*P<0.05*).

During thawing of carp, the nitrogen from aminoacids, **NAA** (g %), constantly increased recording a maximum at 30 hours, that is 5 times higher than the blank sample (frozen fish – Table 1), then it has

decreased until 48 hours, reaching 2.8 times higher than blank.

At 48 hours of thawing, the pH of the ***catfish*** samples recorded an increase by 1.45 pH units, compared to the blank (frozen fish – Table 1). Significant increases of pH values have been recorded at 9, 15, 30 and 42 hours of thawing (*P<0.05*).

During thawing of catfish, **AN** (mg %) increased by 34.3 times compared with the blank (frozen fish), the largest increase being recorded in the range 9-15 hours (*P<0.05*).

During catfish thawing, **NAA (g %)** increased steadily, recording a maximum at 30 hours (5.4 times higher than blank), then decreased until 48 hours, reaching 2.7 times higher than blank.

Compared to the blank (frozen fish - Table 1), at 48 hours of ***mackerel*** thawing, its pH recorded an increase by 1.20 pH units. Significant increases of pH values were recorded at 9, 21, and 30 hours of thawing (*P<0.05*).

After 48 hours from the start of thawing, **AN** (mg %) of mackerel increased 16.2 times compared to the blank (frozen fish - Table 1), the largest increase being recorded in the range 9-15 hours (*P<0.05*).

During mackerel thawing, **NAA** (g %) increased constant, recording a maximum at 30 hours (5.8 times higher than blank), then decreased, being at 48 hours 3.8 times higher than blank.

At 48 hours of thawing, the pH of the ***hake*** samples recorded an increase by 1.32 pH units, compared with the blank. Significant increases of pH values were recorded at 9, 15, 30, and 42 hours of thawing (*P<0.05*).

During hake thawing, **AN** (mg %) increased by 25 times compared with the blank (frozen fish), the largest increase being recorded in the range 9-15 hours (*P<0.05*).

During thawing of hake, **NAA** (g %) increased steadily, reaching a maximum at 30 hours (10.5 times higher than blank), then decreased until 48 hours, being, finally, 6 times higher than blank.

In Figure 1 is shown the comparative evolution of amino nitrogen (AN) in fish samples during thawing.

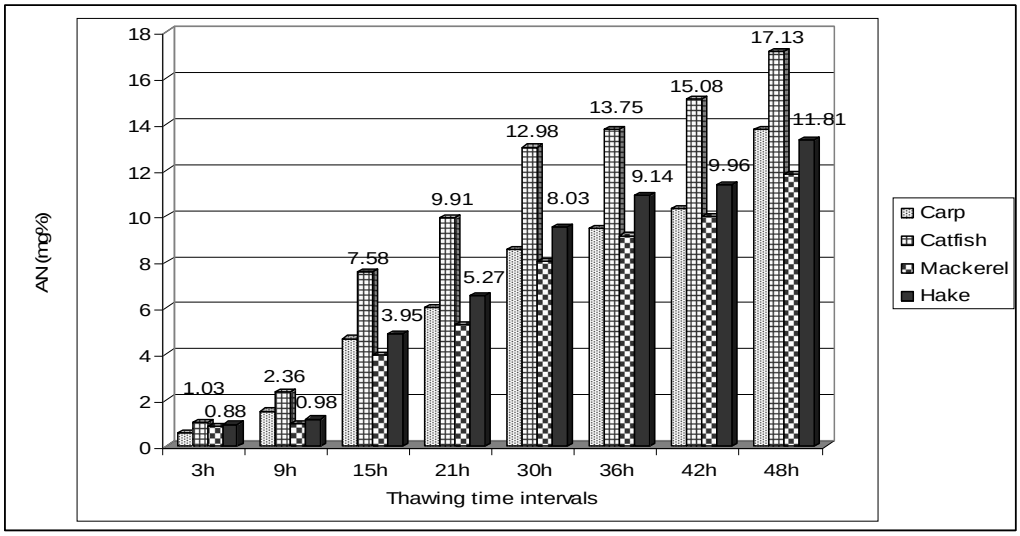

Fig. 1. The comparative evolution of AN (mg %) in fish samples during thawing

As seen in Fig 1, the amino nitrogen (AN) increased, during slow thawing, in all analysed fish samples, the lowest values being registered in mackerel (0.88 – 11.81 mg %), and the greatest ones in catfish (1.03-17.13 mg %).

The amino nitrogen (trimethylamine) values can indicate the freshness degree of the fish

meat. According to Castell and Triggs, cited by [14], 0-1 mg % amino nitrogen (trimethylamine) indicates fresh fish, 1-5 mg % relative fresh fish, and 5 mg % altered fish.

From Fig. 1 it can be seen that, at 3 hours of thawing, AN values show, in all cases, fresh fish, but at 9 hours only mackerel was still fresh, while the others were already fishes with relative freshness, with smaller values and close in carp and hake, and much greater in catfish (*P<0.05*). Starting with 15 hours of thawing, in all analyzed fishes the amino nitrogen values have shown an altered state.

The Fig. 2 reproduces the comparative evolution of nitrogen from aminoacids (NAA) in fish samples during slow thawing.

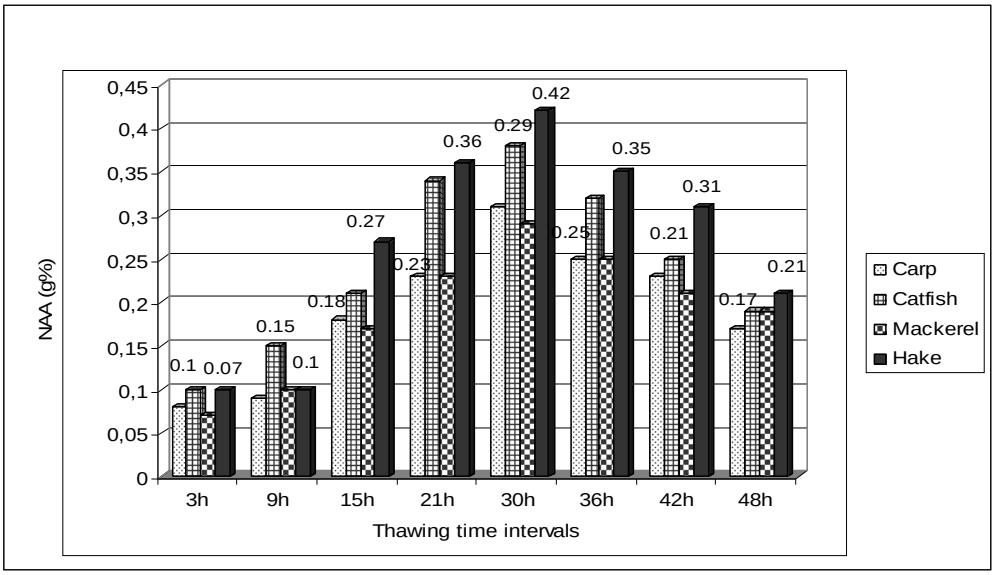

Fig. 2. The comparative evolution of NAA (g %) in fish samples during thawing

As seen in the graph, for all fish samples the nitrogen from aminoacids (NAA) values had a similar evolution, that is a significant increase up to 30 hours of thawing, compared to frozen fishes (*P<0.05*). At 30 hours, it followed a constant decrease of NAA up to the end of analyzed period (48 hours).

According to Beschea and Toma [14], NAA content more than 0,1 g per 100 g of product is usually associated with the beginning of fish alteration.

The evolution of NAA during thawing (Table 2 and Fig. 2), highlights, at 9 hours, a beginning of alteration only in catfish and hake, and at 15 hours in all the fish samples analyzed. At 30 hours of thawing, hake registered the highest NAA value, compared to frozen sample, and to carp, catfish and mackerel with close values (*P<0.05*). Although NAA values decreased, steadily, in all fish samples between 30 and 48 hours of slow thawing, in the end that values were significant higher than those of frozen fishes.

In order to extend the keeping quality of fish it should lower their body temperature [16], 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 [6, 7].

The deterioration of quality of both wild and farmed fish species is mainly due to action of intrinsic enzymes and microbes [8, 9, 10].

The decrease of NAA values after 30 hours of thawing can be attributed to the conversion of aminoacids (released by proteolysis) in subcomponents, by means

of biochemical processes like: decarboxylation, deamination and desulfuration.

4. Conclusions

The evolution of amino nitrogen, nitrogen from aminoacids and pH values in frozen fishes (carp, catfish, mackerel, and hake), during 48 hours of slow thawing at room temperature (20-22°C), showed differences between these species.

The amino nitrogen values indicated a fresh fish at 3 hours of thawing, in all cases, a fish with relative freshness at 9 hours of thawing (except mackerel which was still fresh), and an altered state of all fishes after 15 hours of thawing.

During fish slow thawing, the evolution of nitrogen from aminoacids has emphasized the beginning of alteration, at 9 hours, only in catfish and hake, and at 15 hours in all analyzed fishes.

Slow thawing at room temperature have proved that, from the four frozen fish species analyzed, catfish and hake had the highest spoilage speed, and mackerel the lowest one.

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