THE EVOLUTION OF SOME NITROGEN COMPOUNDS DURING SLOW THAWING OF FOUR FROZEN FISH SPECIES

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Abstract: The purpose of this work was to search the evolution of the amino nitrogen (mg AN %) and nitrogen from aminoacids (g NAA %) in four fish species during 36 hours of slow thawing (at +20..+22°C) to see which species have a higher spoilage speed in this conditions. The biological material was represented by frozen fishes (Norwegian herring, mackerel and salmon, and Romanian trout) which were subjected to slow thawing at room temperature (+20..+22°C), analyzing, at certain intervals, amino nitrogen (mg NA %), nitrogen from aminoacids (g NAA %) and pH values. 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. The nitrogen from aminoacids was evaluated according to Sörensen method, based on blocking the amino group of the aminoacids with formaldehyde, forming methylene-derivatives with acid reaction which were determined titrimetrically, and the pH was determined with a digital pH-meter type Hanna. The evolution of nitrogen compounds in frozen fishes during 36 hours of thawing at +20..+22°C, has shown differences between these species. So, the amino nitrogen values have indicated: fresh fish at 3 hours of thawing, fish with relative freshness at 6 hours of thawing, with lower values (and close) in herring, mackerel and salmon, and much greater in trout; an altered state of all fishes after 9 hours of thawing. The evolution of nitrogen from aminoacids values during the thawing fish has highlighted the beginning of alteration: at 3 hours only in trout; at 6 hours in herring, salmon and trout, and after 9 hours in all examined fishes.

INTRODUCTION

The diversity of fish species is very large. The rate of fish perishability 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 (Mehta et al., 2011; Hsieh and Kinsella 1989; Pigott and Tucker 1987). In order to extend the keeping quality of fish it should lower their body temperature (Pigott and Tucker 1990).

According to Bennour et al. (1991), Nunes et al. (1992), Olafsdottir et al. (1997), 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.

During the freezing, thawing, and frozen storage of fish muscle some deteriorations occur, including changes in flavour, colour, odour, and texture (Matsumoto, 1980). The freeze-thaw process caused fibre distortion and an increased gap between fibres in whole tiger shrimp (Boonsumrej et al., 2007). According to Xia et al. (2009; 2010), 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.

During the frozen storage of meat, protein and lipid oxidation occur and have an important influence on product acceptability (Eymard et al., 2009). 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 (Kong et al., 2013).

Though, according to Campo-Deaño et al. (2009), 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.

In this paper it has searched the evolution of two nitrogen compounds during slow thawing (+20..+22°C) of four frozen fish species to see which species have a higher spoilage speed in this conditions.

MATERIALS AND METHODS

The biological material was represented by four fish species (Norwegian herring, mackerel and salmon and Romanian trout), purchased chilled from supermarket and then kept frozen (at -29°C) for 1 month.

The fish samples was subjected to slow thawing at room temperature (+20..+22°C), analyzing, at certain intervals, amino nitrogen (mg NA %), nitrogen from aminoacids (g NAA %) and pH values.

The amino nitrogen (mg AN %) was determined by the difference between the nitrogen content of volatile bases and the nitrogen content of the ammonia and primary amines (Beschea and Toma, 1984).

The nitrogen from aminoacids (g NAA %) was evaluated according to Sörensen method, based on blocking the amino group of the aminoacids with formaldehyde, forming methylene-derivatives, with acid reaction, which were determined titrimetrically (Beschea and Toma, 1984).

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

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.

RESULTS AND DISCUSSIONS

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

Table 1. Biochemical indices values in frozen fish samples

Fish species Biochemical indices	Herring	Mackerel	Salmon	Trout
pH	6.40	6.35	6.40	6.45
AN (mg la 100 g produs)	0.82±0.01	0.78±0.05	0.73±0.02	0.80±0.03
NAA (g la 100 g produs)	0.06±0.00	0.08±0.01	0.07±0.00	0.08±0.02

As seen, the values of the analyzed indices show small differences between the four species of analyzed fish.

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

Table 2. Biochemical indices values during fish thawing

Test times		**3 h**	**6 h**	**9 h**	**12 h**	**24 h**	**27 h**	**30 h**	**36 h**
Samples	**Tests**								
F1	pH	6.62	6.80	6.85	6.90	6.90	7.01	7.09	7.15
	AN (mg %)	0.96 ±0.08	1.9 ±0.05	6.5 ±1.04	8.3 ±1.89	9.6 ±0.51	9.9 ±0.03	10.8 ±1.16	11.4 ±0.61
	NAA (g %)	0.08 ±0.03	0.12 ±0.01	0.21 ±0.02	0.29 ±0.00	0.23 ±0.04	0.20 ±0.06	0.17 ±0.02	0.14 ±0.01
F2	pH	6.55	6.64	6.70	6.82	6.90	6.98	7.11	7.15
	AN (mg %)	0.90 ±0.00	1.56 ±0.05	7.25 ±1.14	9.7 ±0.44	10.9 ±1.67	11.5 ±0.85	12.3 ±1.73	12.9 ±0.09
	NAA (g %)	0.09 ±0.02	0.10 ±0.05	0.19 ±0.01	0.27 ±0.02	0.29 ±0.04	0.21 ±0.00	0.14 ±0.04	0.11 ±0.02
F3	pH	6.53	6.69	6.70	6.71	6.85	7.04	7.04	7.19
	AN (mg %)	0.85 ±0.07	1.8 ±0.05	7.5 ±0.14	10.2 ±1.04	12.5 ±0.08	12.8 ±1.81	13.2 ±1.52	13.8 ±0.97
	NAA (g %)	0.09 ±0.00	0.14 ±0.05	0.19 ±0.01	0.26 ±0.07	0.21 ±0.04	0.19 ±0.02	0.17 ±0.04	0.17 ±0.03
F4	pH	6.65	6.80	6.85	6.90	7.05	7.09	7.15	7.33
	AN (mg %)	0.95 ±0.07	3.1 ±0.05	8.15 ±1.74	13.7 ±0.89	14.8 ±1.19	15.2 ±0.44	16.6 ±0.58	17.3 ±1.06

Test times		3 h	6 h	9 h	12 h	24 h	27 h	30 h	36 h
Samples	Tests								
	NAA (g %)	0.12 ±0.04	0.29 ±0.02	0.38 ±0.00	0.35 ±0.06	0.36 ±0.01	0.32 ±0.03	0.25 ±0.07	0.22 ±0.05

F1=herring; F2=mackerel; F3=salmon; F4=trout; AN=amino nitrogen; NAA=nitrogen from aminoacids

As seen in Table 2, at the end of the analyzed period, the pH of the herring samples has recorded an increase by 0.75 pH units, compared with the blank (frozen fish - Table 1). The largest increases were recorded in the ranges: 0-3 hours (0.22 pH units) and 3-6 hours (0.18 pH units).

After 36 hours from the beginning of thawing, the amino nitrogen (mg AN %) of herring has increased by 13.9 times compared with the blank (frozen fish - Table 1), the largest increase being recorded in the range 6-9 hours ($p < 0.05$).

The nitrogen from aminoacids (g NAA %) during thawing of herring has increased steadily, recording a maximum at 12 hours (4.8 times higher than blank), then has decreased until the end of the study period (36 hours).

After 36 hours of thawing, the pH of the mackerel samples has recorded an increase by 0.80 pH units, compared with the blank. The largest increases were recorded in the ranges: 0-3 hours (0.20 pH units) and 9-12 hours (0.12 pH units).

During thawing, the amino nitrogen (mg AN %) of mackerel has increased by 16.5 times compared with the blank (frozen fish), the largest increase being recorded in the range 6-9 hours ($p < 0.05$).

The nitrogen from aminoacids (g NAA %) during thawing of mackerel has increased steadily, recording a maximum at 24 hours (3.6 times higher than blank), then has decreased until 36 hours.

Compared with the blank (frozen fish - Table 1), after 36 hours of thawing the pH of the salmon samples has recorded an increase by 0.79 pH units. The largest increases were recorded in the ranges: 24-27 hours (0.19 pH units), and 3-6 hours (0.16 pH units).

After 36 hours from the beginning of thawing, the amino nitrogen (mg AN %) of salmon has increased by 18.9 times compared with the blank (frozen fish - Table 1), the largest increase being recorded in the range 6-9 hours ($p < 0.05$).

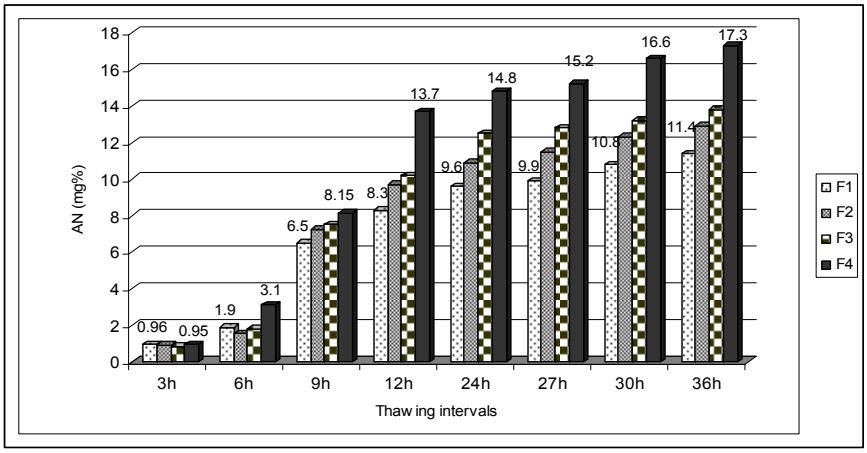
The nitrogen from aminoacids (g NAA %) during thawing of salmon has increased steadily, recording a maximum at 12 hours (3.7 times higher than blank), then has slightly decreased until the end of the study period (36 hours).

After 36 hours of thawing, the pH of the trout samples has recorded an increase by 0.88 pH units, compared with the blank. The largest increases were recorded in the ranges: 0-3 hours (0.20 pH units) and 30-36 hours (0.18 pH units).

During thawing, the amino nitrogen (mg AN %) of trout has increased by 21.6 times compared with the blank (frozen fish), the largest increase being recorded in the range 3-6 hours ($p < 0.05$).

The nitrogen from aminoacids (g NAA %) during thawing of trout has increased steadily, recording a maximum at 9 hours (4.7 times higher than blank), then has decreased until 36 hours.

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



F1=herring; F2=mackerel; F3=salmon; F4=trout

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

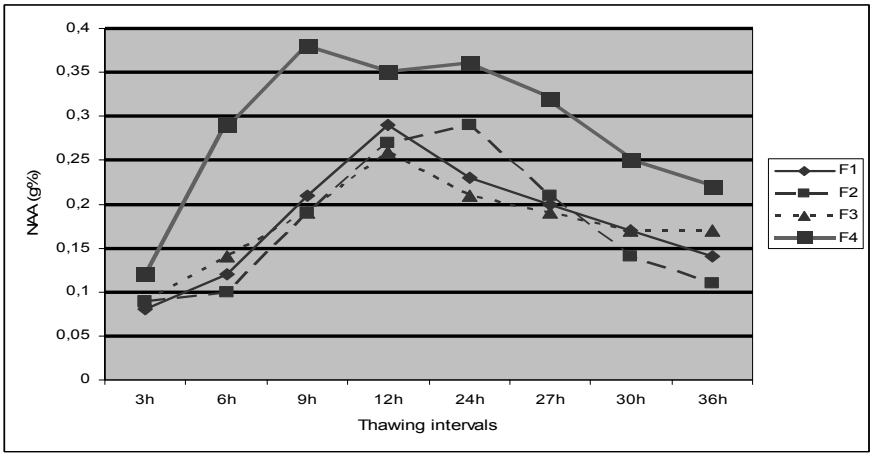
As can be seen from the graph, since the early hours of thawing process, the amino nitrogen increased, reaching, after 9 hours, values between 6.5% (in herring) and 8.15 (in trout).

Starting with 9 hours and ending with 36 hours, in all analyzed intervals one can observe a steady increase of this biochemical index in all four analyzed species, the hierarchy of amino nitrogen values being as follows: trout > salmon > mackerel > herring ($p < 0.05$). In the same period (9-36 hours), the difference between the highest value of amino nitrogen (in trout) and lowest one (in herring) increased, doubling or even tripling.

After Castell and Triggs (cited by Beschea and Toma, 1984), the fish amino nitrogen (trimethylamine) values are as follows: 0-1 mg % for fresh fish, 1-5 mg % for relative fresh fish, and 5 mg % for altered fish.

From the Figure 1 it observes that, if at 3 hours of thawing the amino nitrogen values show, in all cases, fresh fish, at 6 hours the amino nitrogen values indicates, in all cases, fishes with relative freshness, with smaller values and close in herring, mackerel and salmon ($p < 0.05$) and much greater in trout. Starting with 9 hours of thawing, in all analyzed fishes the amino nitrogen values have shown an altered state.

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



F1=herring; F2=mackerel; F3=salmon; F4=trout

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

As seen from the Figure 2, the nitrogen from aminoacids (NAA) values have evolved differently in herring, mackerel and salmon compared with trout. Thus, in the first three species mentioned above, throughout the thawing (3-36 hours), the values of samples were close ($p < 0.05$), with maximum at 12 hours for herring and salmon, and at 24 hours for mackerel.

In trout, the values were much higher, with a maximum at 9 hours ($p < 0.05$). The decrease of NAA values once with increasing of the thawing period can be attributed to the conversion of aminoacids (released by proteolysis) within subcomponents by means of decarboxylation, deamination, etc.

For fish, NAA content more than 0,1 g per 100 g of product is usually associated with the beginning of alteration (Beschea and Toma, 1984). Following the evolution of NAA during thawing (Table 2 and Figure 2), it can see, at 3 hours, a beginning of alteration only in trout, at 6 hours in herring, salmon and trout, and after 9 hours in all analyzed fishes.

CONCLUSIONS

The evolution of some nitrogen compounds in frozen fishes (herring, mackerel, salmon and trout), during 36 hours of thawing at +20..+22°C, has shown differences between these species, corresponding to the different alteration speeds of fishes.

During thawing of the four fish species, the amino nitrogen values have indicated:

- fresh fish at 3 hours of thawing;
- fish with relative freshness at 6 hours of thawing, with lower values (and close) in herring, mackerel and salmon, and much greater in trout;
- an altered state of all fishes after 9 hours of thawing.

The evolution of nitrogen from aminoacids values during the thawing fish has highlighted the beginning of alteration: at 3 hours only in trout, at 6 hours in herring, salmon and trout, and after 9 hours in all examined fishes.

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