Fisk. Dir. Skr., Ser. Ernæring, Vol. II, no. 1, s. 7–24 (1981).

THE NON-PROTEIN NITROGENOUS COMPOUNDS OF FISH USED IN MEAL PRODUCTION, WITH SPECIAL REFERENCE TO CAPELIN (*Mallotus villosus*)

By

JOHS. KJOSBAKKEN and HELGE LARSEN

Department of Biochemistry

The Norwegian Institute of Technology

N-7034 Trondheim-NTH, Norway.

ABSTRACT

5 samples of capelin, 4 samples of herring and 1 sample of mackerel, caught at different times of the year, were analyzed for non-protein nitrogenous compounds within 1 to 24 hours after capture. Whole fish, white muscle, red muscle, liver, roe, soft roe and heart were analyzed for creatine, trimethylamine oxide (TMAO), trimethylamine, taurine and histidine. The white muscle was also analyzed for free amino acids, anserine, ammonia, nucleotides and nucleotide components. The results are given in tables. In capelin muscle TMAO is the dominating non-protein nitrogenous compound followed by creatine; these two compounds make up about 70% of total non-protein nitrogenous compounds. In herring muscle TMAO and creatine contribute about equally to the content of non-protein nitrogenous compounds; together they make up about 65%. In mackerel muscle histidine, in addition to TMAO and creatine, contributes substantially to the non-protein nitrogenous compounds; together the three compounds account for more than 70%. The capelin muscle contains anserine and very little free histidine which supports on a chemical basis the asserted relationship of the capelin to the salmon-like fishes.

INTRODUCTION

A study on the biochemistry of bacterial spoilage of fish stored in bulk prior to reduction to meal and oil, has been pursued in our department for some time (STRØM and LARSEN, 1979; KJOSBAKKEN et al., 1980). As part of this study we have assayed the chemical components of the fish in the fresh state, *i.e.* at the start of the storage period and before a significant spoilage had taken place. Only little information of this kind has been published for capelin (*Mallotus villosus*) despite its economic importance. In the present paper we report on the contents of the non-protein nitrogenous compounds in capelin caught in Northern Norway and the Barents Sea at different times of the year. For comparative purposes we include some of our data on herring (*Clupea harengus*) and mackerel (*Scomber scombrus*) caught in Western Corway and the North Sea. Additional data and detailed discussions on analytical procedures are given by KJOSBAKKEN (1970).

Table 1. Catch information on the fish samples.

Sample-Designation	Caught	Hours before processing	Remarks
Capelin, Febr.	Febr. 22, '68 in Varangerfjord	18	Start of spawning season
Capelin, March	March 11, '68 northheast of Vardø	8	Middle of spawning season
Capelin, April	April 17, '68 north of Vardø	24	End of spawning season
Capelin I, Nov.	Nov. 5, '68 at 73°25'N, 33°40'E	1	Barents Sea
Capelin II, Nov.	Nov. 2, '68 at 73°55'N, 30°22'E	1	Barents Sea
Herring, Sept.	Sept. 8, '67 at Vallersund	10–12	Summer herring, kept alive 3½ days in the purse seine
Herring, Dec.	Dec. 6. '67 at Levanger	14–15	Fjord (young) herring
Herring, March	March 20, '68 at Buadjupet	18	Winter (spawning) herring
Herring, April	April 30, '68 southwest of Egersund	15	North Sea
Mackerel, April	April 30, '68 southwest of Egersund	8	North Sea

MATERIALS AND METHODS

Sample preparation

Relevant data for the fish samples used in the present study are listed in Table 1. Capelin samples I and II, Nov., were caught by trawl by R/V «Johan Hjort» and samples frozen within 1 hour and stored at –25 to –35°C until processed for analysis. The other samples were taken from the catch of commercial purse seiners shortly after landing. They were all found «fresh» by sensorial evaluation. Single specimens were dissected and samples of the following tissues collected: white muscle, red muscle, liver, hard roe, soft roe and heart. The samples, single or pooled, were immediately immersed in acid for extraction of non-protein nitrogenous compounds. Some samples from the commercial catches were frozen and stored at –25 to –35°C until processed for analysis.

Extraction procedures

When necessary the samples were minced in a meat grinder and thoroughly mixed before extraction.

Trichloroacetic acid (TCA) extracts. 20 g of tissue was extracted twice with 40 ml 10% (w/v) TCA using the Ultraturrax TP 18/2 Homogenizer. The filtrates were pooled and the second filter cake washed on the filter with 10% (w/v) TCA to a total volume of 100 ml and stored at 4°C.

Perchloric acid (PCA) extracts. 10 g of tissue was extracted twice with 40 ml 0.6 N perchloric acid using the Ultraturrax. The filtrates were pooled and the second filter cake washed on the filter with 0.6 N perchloric acid to give a total volume of about 90 ml. 5 N KOH was added to give pH 6.5, then water to 100 ml. After 3 hours at 4°C $KClO_4$ was removed by filtration (JONES and MURRAY, 1962). The extract was stored at –35°C. For the determination of ATP and ADP the extraction was carried out at 4°C, otherwise at room temperature.

Picric acid (PA) extracts. 5 g of tissue was extracted twice with 40 ml 1% (w/v) picric acid using the Ultraturrax. The filtrates were pooled and the second filter cake washed on the filter with 1% (w/v) picric acid to give a total volume of 100 ml. Picric acid was removed by passing 40 ml of the extract through a column (2 cm diam. × 3 cm) of Dowex 2 × 8 Cl^- (200–400 mesh) and eluting the non-protein nitrogenous compounds with 4 × 10 ml 0.02 N HCl. The eluate was evaporated to dryness in vacuum at 20°C, and the residue dissolved in 5 ml 0.2 N Na citrate buffer pH 2.2 which was stored at –35°C.

Analytical procedures

Creatine was determined in the TCA extracts using the colorimetric method of JAFFÉ as described by HUGHES (1960). Creatine phosphate was determined according to ENNOR and ROSENBERG (1952).

Trimethylamine oxide (TMAO), *trimethylamine* (TMA) and *ammonia* were determined in the TCA extracts using the microdiffusion technique of CONWAY and BYRNE (1933). For the determination of TMA formaldehyde was added to bind ammonia (HJORTH-HANSEN and BAKKEN, 1947). TMAO was determined as TMA after reduction with $TiCl_3$ as described by RONOLD and JAKOBSEN (1947).

Free amino acids, nucleosides and free purines were determined in the citrate buffer solutions from the PA extracts using the Beckman Model 120C Amino Acid Analyzer and conventional procedure. Also anserine and other dipeptides were determined by this method. For the registration of nucleosides and purines a LKB Uvicord Model 4701A was attached to the Beckman instrument as described by BONNELYCKE, DUS and MILLER (1969). Separate analyses were carried out for histidine and taurine in the TCA extracts. For this purpose the TCA was removed from the extracts by ether extraction (GARVIN, 1960), and histidine determined colorimetrically with PAULY's reagent as described by SNELL and SNELL (1937) and taurine colorimetrically after separation by ion exchange as described by GARVIN (1960).

Nucleotides were determined in the PCA extracts. The samples were added to a column (1.3 cm diam. × 18 cm) of Dowex 1 × 8 formate (200–400 mesh), and nucleotides effectively fractionated by elution with a solution of increasing concentration of formate in water and decreasing pH as described by JONES and MURRAY (1962). UV-absorbing compounds in the eluate were registered with a LKB Uvicord Model 4701A. The fractions were evaporated to dryness in vacuum at 20°C, the residues dissolved in 0.01 N HCl and the nucleotides determined spectrophotometrically in 0.1 M acetate buffer pH 4.4 at 260 nm (Zeiss Spektralphotometer Model PMQ II) according to HORI (1967).

Total nitrogen was determined in the TCA extracts by the Kjeldahl method (LEGGET BAILEY, 1967).

The analytical methods were tested on known compounds after adding them to extracts and/or tissues. The recoveries were satisfactory.

RESULTS

Creatine

The creatine content of whole fish seems to be at about the same level in the 3 fishes (Table 2). The highest contents were found in white muscle, considerably less in red muscle and heart, and relatively little in liver, hard roe and soft roe. The creatine content of the white muscle was somewhat lower in capelin than in herring and mackerel. The data do not reveal any seasonal change.

The creatine values for white muscle of herring were higher than those reported by HUGHES (1960) for herring (*Clupea harengus*) caught near Scotland,

Table 2. Creatine content (μmol/g wet weight) of capelin, herring and mackerel.

	Whole fish	White muscle	Red muscle	Liver	Roe	Soft roe	Heart
Capelin, Febr.		34.1±1.8(5)[1])	19.1(5)	4.8(25)	7.5.(1)	10.0(1)	16.5(50)
Capelin, March	16.7±1.9(5)				6.6±0.7(5)	8.3(8)	
Capelin, April	21.5±1.4(5)	32.1±3.4(9)	18.6(10)	6.6(50)	6.6(1)		18.5(50)
Capelin, I, Nov.		30.3±0.5(5)					
Capelin, II, Nov.	22.0±1.3(5)	34.4±0.7(5)					
Herring, March	24.8±0.5(5)	49.3±1.0(5)	22.2±2.4(5)	3.0(10)	5.2±0.4(5)	12.0±1.2(5)	19.4(20)
Herring, April		52.8±1.5(5)	21.1±1.5(5)				
Herring, Sept.		57.0±2.7(5)	32.1±2.0(5)	13.9(20)			14.6(35)
Herring, Dec.	26.1±1.6(5)	46.7±2.5(5)	24.5±3.1(5)	4.2(15)	5.5±0.8(4)	10.0±2.9(4)	17.0(25)
Mackerel, April	23.3±0.8(5)	41.2±1.3(6)	18.9±2.7(6)	5.5±2.4(5)	5.8±0.3(5)	10.2±0.55(5)	14.5(20)

[1]) ± denotes standard deviation and appending figures in brackets the number of individuals analyzed. Where no ± is given the appending figures in brackets denote the number of samples pooled for analysis.

Table 3. Trimethylamine oxide content (μmol/g wet weight) of capelin, herring and mackerel.

	Whole fish	White muscle	Red muscle	Liver	Roe	Soft roe	Heart
Capelin, Febr.		55.8±3.3(5)[1])	44.3(5)	22.8(25)	14.8(1)	22.7(1)	33.9(50)
Capelin, March	36.4±4.6(5)				16.5±5.0(5)	17.1(8)	
Capelin, April	35.1±2.2(5)	49.0±4.7(9)	37.6(10)	15.7(50)	11.1(1)		33.6(50)
Capelin, I, Nov.		56.2±1.9(5)					
Capelin, II, Nov.	47.0±1.4(5)	64.3±5.3(5)					
Herring, March	30.8±5.5(5)	41.2±2.4(5)	39.1±2.0(5)	11.2(10)	12.8±0.7(5)	55.7±3.5(5)	25.5(20)
Herring, April		32.9±4.7(5)	27.9±4.0(5)				
Herring, Sept.		20.9±1.5(5)	9.2±0.5(5)	1.5(20)			2.5(35)
Herring, Dec.	34.2±3.1(5)	47.1±5.7(5)	25.2±0.5(5)	9.9(15)	15.5±1.4(4)	21.1±8.5(4)	21.2(25)
Mackerel, April	18.9±0.7(5)	27.5±2.4(6)	33.1±2.4(6)	2.4±1.6(5)	4.8±0.8(5)	30.3±5.4(5)	20.5(20)

[1]) See Table 2.

but agree well with other data on herring reviewed by SHEWAN (1951). Our values on white muscle of the Atlantic mackerel (*Scomber scombrus*) agree quite well with those given by SAKAGUCHI and SIMIDU (1965) for the Pacific mackerel (*Scomber japonicus*).

Creatine contents found in white muscle tissue of 5 specimens of capelin (Febr.) males and 5 specimens of capelin (Febr.) females did not reveal any difference between the sexes.

Creatine phosphate was not found in any of the samples. This was not surprising since it is well known that creatine phosphate is rapidly hydrolyzed in fish *post mortem* (NAZIR and MAGAR, 1963).

Trimethylamine oxide

It has been reported that herring has a considerably lower content of TMAO in the summer than in the winter (RONOLD and JAKOBSEN, 1947; HUGHES, 1959a), and our data agree with these earlier findings (Table 3). For capelin the data indicate that during the winter and spawning season (Nov.–April) the TMAO content fluctuates little and is somewhat higher than that normally found for herring during the winter season. This was also observed by T. STRØM (personal communication). For capelin we have no data for the summer period.

5 specimens of capelin (Febr.) males and females were analyzed for the TMAO content of the white muscle. No significant difference between the sexes was registered.

White muscle was relatively rich in TMAO. In most of the samples from capelin and herring the TMAO contents of white muscle were higher than in the other tissues analyzed, the one exception was soft roe of the herring caught in March which had a very high TMAO content. The very low TMAO values for red muscle, liver and heart in September caught herring are remarkable.

The one sample of mackerel analyzed (April) showed similar TMAO contents for white muscle, red muscle and soft roe. The heart had a lower content, and very low values were found for liver and roe.

Trimethylamine

The samples analyzed for TMAO (Table 3) were also analyzed for TMA. The values for TMA were negligible both for whole herring and the various herring tissues. One sample of whole capelin (April) showed a TMA content of 3.4 ± 0.7 µmol/g wet weight (5 individuals analyzed). All other samples had either a negligible content or less than 1 µmol/g. Newly caught capelin (whole fish) has been reported to contain 0.1–0.4 µmol/g TMA (SHAW and BOTTA, 1975a, b; BOTTA *et al.*, 1978). We have no strong evidence to claim that TMA is

a component of the tissues of living fish. The small amounts of TMA registered may be due to bacterial reduction of TMAO between the time of capture and sampling, and/or due to bacterial activity in the gut of the living fish, as suggested by RONOLD and JAKOBSEN (1947) for corresponding findings in herring. The latter suggestion may also apply to our findings in mackerel (April) where a small amount of TMA (1.6 ± 0.2 μmol/g, 5 specimens analyzed) was registered in the whole fish, but negligible amounts in white and red muscle, hard and soft roe, liver and heart.

Ammonia

The samples analyzed for TMAO (Table 3) and TMA were also analyzed for ammonia. Values in the range 4–10 μmol/g wet weight were found. No particulars were noticed concerning type of fish, type of tissue, or season.

It is well known that ATP and ADP of tissues is broken down rapidly by endogenous enzymatic activity *post mortem*, with the formation of inosine monophosphate (IMP) and liberation of ammonia. These conversions have also been shown to occur rapidly after capture in fish (TARR, 1966) and recently specifically in capelin (*Mallotus villosus*) (SHAW and BOTTA, 1975a). In the present work we analyzed for the breakdown products of ATP and ADP (*i.e.* IMP, inosine, hypoxanthine; Table 9). From the figures obtained an estimate can be made of the amounts of ammonia liberated from ATP and ADP. The results are given in Table 4 and show that the values for ammonia determined by direct analysis correspond well with those estimated from the degradation products of ATP and ADP. The results therefore support the contention that the small amounts of ammonia found in fish muscle *post mortem*, and before a bacterial production sets in, arise mainly from an endogenous deamination of the adenine of ATP and ADP.

Table 4. Ammonia (μmol/g wet weight) in white muscle of capelin, herring and mackerel, determined by direct analysis and estimated from degradation products of ATP and ADP (Table 9).

	Direct analysis	Estimate from ATP/ADP degradation
Capelin, March	5.7±0.7(5)[1])	4.6
Capelin, April	7.1±0.7(9)	4.1
Capelin, II, Nov.	6.9±0.6(5)	5.8
Herring, March	10.6±1.2(5)	7.2
Herring, Dec.	7.6±1.2(5)	11.1
Mackerel, April	7.1±1.2(5)	8.2

[1]) See Table 2.

Taurine

The taurine contents of whole fish samples were appreciable and about the same in the three species of fish, but varied much from one type of tissue to another (Table 5). The white muscle had a low content compared to the other tissues. The red muscle, especially of herring and mackerel, had a remarkably higher content, and so did the heart of all three species. A content of about 60 μmol/g wet weight corresponds roughly to about 4% of the dry weight.

The herring caught in September differed markedly from that caught during winter and spring. The taurine contents of the white muscle were higher whereas the contents of the red muscle and the heart were lower. This may reflect a seasonal change, as does the contents of TMAO.

No significant difference was found in the taurine content of white muscle of capelin (Febr.) males and females upon analysis of 5 specimens of each sex.

HUGHES (1959b) reported the taurine content of herring (*Clupea harengus*) muscle (7–13 μmol/g), but did not distinguish between white and red muscle tissue. However, a profound difference between white muscle (8 μmol/g) and red muscle (38 μmol/g) was reported for anchovy (*Engraulis japonicus*) (ARAKAKI and SUYAMA, 1966). Information about the distribution of taurine in fish tissues is otherwise scarce. SAKAGUCHI and SIMIDU (1965) reported a taurine content of only 0.2–0.7 μmol/g in the white muscle of Pacific mackerel (*Scomber japonicus*).

Histidine

The histidine content in capelin was low, and there was no tendency to an accumulation in any one of the special tissues analyzed (Table 6). Somewhat higher amounts were found in herring, and appreciable amounts in the September caught herring. Relatively high amounts of free histidine were found in the sample of mackerel, the histidine was mainly concentrated in the white muscle.

There was no significant difference between males and females of capelin (Febr.) upon analysis of 5 specimens of each sex.

The values given in Table 6 agree well with those of HUGHES (1959b, 1964) on white and red muscle of herring (*Clupea harengus*). SAKAGUCHI and SIMIDU (1965) reported a content of free histidine in muscle tissue of Pacific mackerel (*Scomber japonicus*) about double the values found for Atlantic mackerel (*Scomber scombrus*) in the present work.

Table 5. Taurine content (μmol/g wet weight) of capelin, herring and mackerel.

	Whole fish	White muscle	Red muscle	Liver	Roe	Soft roe	Heart
Capelin, Febr.		7.2±0.5(5)[1)]	18.6(5)	14.2(25)	16.2(1)	18.4(1)	52.5(50)
Capelin, March	12.1±0.6(5)				21.0±2.0(5)	25.1(8)	
Capelin, April	12.5±0.5(5)	8.0±1.4(9)	21.2(10)	17.2(50)	15.0(1)		52.2(50)
Capelin, I, Nov.		7.5±0.9(5)					
Capelin, II, Nov.	10.4±0.3(5)	7.9±1.5(5)					
Herring, March	13.9±0.9(5)	5.4±0.6(5)	43.5±4.4(5)	29.8(10)	16.2±0.9(5)	18.6±1.5(5)	61.2(20)
Herring, April		4.7±0.4(5)	38.9±3.0(5)				
Herring, Sept.		14.8±1.4(5)	22.2±2.4(5)	27.3(20)			38.3(35)
Herring, Dec.	15.7±2.1(5)	5.8±0.9(5)	44.9±3.0(5)	25.8(15)	18.1±1.7(4)	28.0±5.5(4)	66.0(25)
Mackerel, April	17.0±0.4(4)	3.4±0.6(6)	41.5±3.0(6)	21.7±4.3(5)	36.0±3.5(5)	36.4±0.9(5)	55.7(20)

[1)] See Table 2.

Table 6. Histidine content (μmol/g wet weight) of capelin, herring and mackerel.

	Whole fish	White muscle	Red muscle	Liver	Roe	Soft roe	Heart
Capelin, Febr.		0.4±0.1(5)[1])	0.6(5)	1.2(25)	1.2(1)	1.3(1)	1.4(50)
Capelin, March	1.5±0.1(5)				1.3±0.4(5)	1.0(8)	
Capelin, April	1.6±0.5(5)	0.3±0.1(9)	0.8(10)	0.9(50)	1.7(1)		1.4(50)
Capelin, I, Nov.		0.3±0.1(5)					
Capelin, II, Nov.	1.3±0.2(5)	0.3±0.1(5)					
Herring, March	3.6±0.5(5)	6.2±2.1(5)	2.1±0.4(5)	1.2(10)	1.9±0.3(5)	0.7±0.2(5)	0.7(20)
Herring, April		9.3±1.3(5)	3.8±0.5(5)				
Herring, Sept.		11.2±2.4(5)	7.8±0.8(5)	4.8(20)			4.2(35)
Herring, Dec.	4.3±0.9(5)	6.8±0.9(5)	2.7±0.4(5)	1.1(15)	2.9±0.3(4)	1.6±0.7(4)	1.1(25)
Mackerel, April	15.5±1.0(6)	21.8±2.6(6)	7.3±2.4(6)	2.3±1.1(5)	2.0±0.5(5)	4.5±1.0(5)	1.3(20)

[1]) See Table 2.

Anserine

The anserine (β-alanyl-1-methylhistidine) content of the capelin muscle was considerable, the content of the herring and mackerel muscle negligible (Table 7). Other dipeptides known to be constituents of muscle tissues, such as balenine (β-alanyl-3-methylhistidine) and carnosine (β-alanyl-histidine), and hydrolysis products of anserine, *i.e.* β-alanine and 1-methylhistidine, were detected in the muscle samples in very small amounts.

Other amino acids

Free amino acids of the white muscle of capelin, herring and mackerel were determined by the Amino Acid Analyzer (Table 8). The figures for histidine agreed well with those obtained by direct colorimetric analysis (Table 6), low content in the capelin muscle, a considerable content in the herring muscle and a high content in the mackerel muscle.

None of the common amino acids occurred in spectacular concentrations in the capelin samples; the contents of glutamic acid, glycine and alanine were somewhat higher than those of the others. Lysine, glycine and alanine contents

Table 7. Anserine content (μmol/g wet weight) of capelin, herring and mackerel white muscle.

Capelin, March	7.2±0.7
Capelin, April	3.8±0.5
Capelin II, Nov.	5.6±0.8
Herring, March	<0.1
Herring, Dec.	<0.1
Mackerel, April	<0.1

Figures are average from analyses of 5 specimens. ± denotes standard deviation.

Table 8. Free amino acids (μmol/g wet weight) of capelin, herring and mackerel white muscle.

	Capelin			Herring		Mackerel
	March	April	Nov. II	March	Dec.	April
Histidine	0.1±0.05	0.2±0.05	0.1±0.05	4.8±1.5	6.2±0.8	26.0±3.0
Lysine	0.6±0.2	0.6±0.1	0.5±0.3	3.7±1.0	4.8±1.6	4.0±1.2
Arginine	0.1±0.05	0.1±0.05	0.2±0.1	0.3±0.1	0.4±0.1	0.3±0.1
Aspartic acid	0.2±0.1	0.2±0.1	0.2±0.1	<0.1	<0.1	<0.1
Glutamic acid	2.0±0.3	1.1±0.1	1.4±0.2	1.2±0.3	0.8±0.2	1.1±0.6
Proline	0.4±0.1	0.5±0.1	0.3±0.1	0.4±0.1	0.5±0.1	1.1±0.4
Glycine	1.1±0.2	1.8±0.2	2.1±0.4	5.5±1.2	4.9±0.7	2.7±0.8
Serine	0.5±0.05	0.7±0.05	0.6±0.2	1.1±0.2	1.1±0.1	0.8±0.2
Threonine	0.4±0.1	0.5±0.1	0.5±0.2	0.7±0.1	0.7±0.1	0.6±0.1
Methionine	0.2±0.05	0.3±0.1	0.2±0.1	0.2±0.1	0.2±0.1	0.2±0.05
Alanine	2.5±0.4	2.7±0.3	3.3±0.9	5.1±0.9	7.9±1.0	3.1±0.8
Valine	0.7±0.2	0.7±0.2	0.5±0.1	0.5±0.1	0.7±0.2	0.6±0.05
Leucine	0.8±0.2	0.7±0.1	0.6±0.2	0.4±0.1	0.5±0.1	0.5±0.05
Isoleucine	0.4±0.1	0.4±0.1	0.3±0.1	0.2±0.05	0.4±0.1	0.3±0.05
Phenylalanine	0.2±0.05	0.2±0.05	0.2±0.1	0.1±0.05	0.2±0.05	0.1±0.05
Tyrosine	0.2±0.05	0.2±0.05	0.2±0.1	0.2±0.05	0.2±0.05	0.2±0.1

Figures are average from analysis of 5 specimens.
± denotes standard deviation.

were high in the herring and mackerel samples, but nowhere near the remarkable concentration of histidine in mackerel.

HUGHES (1959b) reported free amino acid contents in the muscle of herring (*Clupea harengus*) taken in British fisheries and some seasonal variations were demonstrated. The data from the present investigation were not sufficiently extensive to evaluate seasonal variations, but agree reasonably well with those of HUGHES. Our data on free amino acids in Atlantic mackerel (*Scomber scombrus*) agree reasonably well with those of SAKAGUCHI and SIMIDU (1965) in Pacific mackerel (*Scomber japonicus*).

Nucleotides and nucleotide components

Nucleotides and nucleotide components were determined in some of the white muscle samples. ATP and ADP were not found in measurable quantities in any of the samples; AMP was just detectable (about 0.1 µmol/g). Inosine monophosphate (IMP), inosine and hypoxanthine were found in the amounts given in Table 9.

ATP and ADP of fish muscle are broken down *post mortem* by endogenous enzymatic activity. The breakdown pathway leads through AMP to IMP and inosine which firstly accumulate in the muscle. Upon prolonged storage a further degradation of IMP and inosine takes place with the formation and accumulation of hypoxanthine (TARR, 1966). These processes have also been shown to occur in capelin (*Mallotus villosus*) (SHAW and BOTTA, 1975a). These authors found accumulation of IMP and hypoxanthine, but not of inosine, in capelin stored in the cold, and concluded that the conversions of ATP and ADP to IMP and of inosine to hypoxanthine were faster than that of IMP to inosine. In the present investigation not only IMP and hypoxanthine, but also inosine, were found in appreciable amounts in all three samples of capelin (Table 9).

SHAW and BOTTA (1975a, b) found that ATP of capelin stored in ice or refrigerated sea water was degraded in the course of 1–2 days. No ATP or ADP was found in any of the present capelin samples, not even in capelin sample II (Nov.), which was processed (frozen) only 1 hour after capture. This indicates a faster breakdown of ATP and ADP in our samples than in those of SHAW and BOTTA. It is also possible that an extensive breakdown of ATP had taken place as a result of the struggle of the fish during capture, or as a result of the freezing process as demonstrated to occur in a carp (exact name not given) (SAITO and ARAI, 1957).

It is worth nothing (Table 9) that the sample of capelin which was processed 24 hours after capture (Capelin, April, Table 1) had an appreciably lower content of IMP and higher content of hypoxanthine than the other capelin samples which were processed more closely after capture. Possibly this

Table 9. Nucleotides and nucleotide components (μmol/g wet weight) of capelin, herring and mackerel white muscle.

	Inosine monophosphate	Inosine	Hypoxanthine	Sum
Capelin, March	3.6±0.4	0.7±0.1	0.3±0.1	4.6±0.4
Capelin, April	1.5±0.3	1.4±0.2	1.2±0.1	4.1±0.4
Capelin II, Nov.	4.2±0.4	1.3±0.1	0.3±0.1	5.8±0.4
Herring, March	4.7±0.3	1.9±0.2	0.6±0.02	7.2±0.4
Herring, Dec.	6.8±0.4	3.5±0.3	0.8±0.2	11.1±0.5
Mackerel, April	6.1±0.6	1.8±0.4	0.3±0.1	8.2±0.7

Figures are average from analysis of 5 specimens.
± denotes standard deviation.

Table 10. Sum of non-protein nitrogenous compounds determined in capelin, herring and mackerel white muscle.

	Capelin		Herring		Mackerel
	April	Nov.	March	Dec.	April
μmol NPNC/g wet weight	115	137	137	151	130
μmol NPN/g wet weight	205	241	272	297	295
mg NPNC/100 g wet weight	1 214	1 484	1 546	1 747	1 649
mg NPN/100 g wet weight	287	337	381	416	413
μmol total (Kjeldahl) NPN/g	234	245	290		296
% N recovery	88±10	98±6	94±5		100±4

NPNC: non-protein nitrogenous compounds.
NPN: non-protein nitrogen (N in NPNC).

Table 11. Non-protein nitrogenous compounds of capelin, herring and mackerel white muscle, in mole percent of their sum in the muscle.

	Capelin		Herring		Mackerel
	April	Nov.	March	Dec.	April
Trimethylamine oxide	42.6	47.0	30.0	31.1	21.1
Creatine	27.9	25.2	35.9	30.9	31.6
Taurine	7.0	5.8	3.9	3.8	2.6
Anserine	3.3	4.1	0	0	0
Histidine	0.2	0.1	3.5	4.1	20.0
Sum other amino acids	9.3	8.1	14.3	15.4	12.0
Sum IMP, In, Hx	3.6	4.2	5.2	7.3	6.3
Ammonia	5.6	4.9	7.1	7.3	6.4

IMP: inosine monophosphate; In: inosine; Hx: hypoxanthine.

reflects the further breakdown of IMP upon storage, a process which has been shown to be enhanced also by freezing (FRAZER *et al.*, 1968).

Assuming for the fish samples in this investigation, that hypoxanthine was not further degraded during the relatively short period from catch to processing the sum of IMP, inosine and hypoxanthine (Table 9) may be taken as a measure of the ATP and ADP content of the living fish. For the mackerel sample this sum was 8.2 μmol/g which is in good agreement with the nucleotide content reported for mackerel (*Scomber scombrus*) (FRASER *et al.*, 1968). The sums found for the capelin samples were considerably lower than those for the herring and mackerel samples, indicating a lower ATP and ADP content in the capelin.

Small amounts (0.03–0.06 μmol/g) of nicotinamide adenine dinucleotide (NAD) were detected in the two samples of herring analyzed (Table 9); no NAD was detected in the capelin and mackerel samples.

No nucleotides, nucleosides, purines or pyrimidines other than those reported above were detected in the fish samples analyzed.

DISCUSSION

Table 10 gives the sums of the values listed in Tables 2–9 for the non-protein nitrogenous compounds analyzed in capelin, herring and mackerel white muscle. The figures do not differ much, but the content of non-protein nitrogenous compounds of capelin and herring is somewhat higher in the late fall than during the spring, and the content of these compounds in herring and mackerel is somewhat higher than in capelin. Table 10 also lists the recoveries of nitrogen in the non-protein nitrogenous compounds, in percentage of total (Kjeldahl) nitrogen extractable in trichloroacetic acid. The recoveries are satisfactory and show that no major N-extractives have been overlooked in the analysis.

MACCALLUM and ADAMS (1969), using essentially the same methods of analysis, found the Newfoundland capelin (*Mallotus villosus*) to contain 236 μmol non-protein nitrogen (NPN) per g wet weight muscle. This agrees well with the figures in Table 10. SHEWAN (1951) reported that herring (*Clupea harengus*) contained 270–290 μmol NPN per g and the mackerel (*Scomber scombrus*) 300–320 μmol NPN per g. These values also agree well with those of Table 10. SAKAGUCHI and SIMIDU (1965), however, found considerably higher values (300–430 μmol NPN per g) for the Pacific mackerel (*Scomber japonicus*), primarily due to the higher content of histidine in this fish.

Table 11 gives, on a molar basis, the contribution as percentages of the various non-protein nitrogenous compounds to the total content of these compounds in capelin, herring and mackerel white muscle. It should be emphasized that the figures depict the situation in the muscle 1–24 hours after

the fish was caught (*i.e.* complete breakdown of ATP and ADP to inosine monophosphate, inosine, hypoxanthine and ammonia), but before any significant bacterial spoilage had taken place. It appears that trimethylamine oxide is the dominating non-protein nitrogenous compound of the muscle in capelin, followed by creatine. These two compounds make up about 70% of total non-protein nitrogenous compounds. In herring muscle TMAO and creatine contribute about equally to the content of non-protein nitrogenous compounds; together they make up about 65%. In mackerel histidine, in addition to TMAO and creatine, contributes substantially to the NPN content; together the three compounds make up more than 70%.

Capelin is biologically classified with the salmon-like fishes (WOLLEBÆK, 1924; JANGAARD, 1974). It is interesting to note that in the Atlantic salmon (*Salmo salar*) were found considerable amounts of anserine and only negligible amounts of free histidine (COWEY *et al.*, 1962; COWEY and PARRY, 1963). The sockeye salmon of the North Pacific (*Oncorhynchus nerka*) also contains anserine, and modest amounts of histidine (WOOD, 1958). The present results, a high content of anserine, and a low content of histidine in the capelin thus support on a chemical basis the relationship of the capelin to the salmonoids.

ACKNOWLEDGEMENT

Our thanks are due to Mrs. Tove Olsen who assisted ably in the analytical work, and to Mr. T. Kvande Pettersen, Mr. K. B. Jørgensen, Mr. T. Monstad who helped taking the fish samples. The work was supported by a grant from The Royal Norwegian Council for Scientific and Industrial Research.

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