

NMR — SPECTROSCOPY TECHNIQUE FOR SALMON FISH SPECIES QUALITY ASSESSMENT

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ABSTRACT

NMR-spectroscopy was used for development of the criteria which characterize the chilled and frozen fish quality. It has been shown that ^1H -NMR experiments can be used as quality factor to measure the concentration of inosine, hypoxanthine and inosine-5'-monophosphate generated during the fish storage. The quality factor is expressed by the K_1 correlates well with the sensory quality of chilled Atlantic salmon (*Salmo Salar*), whereas, quality factor H is more sensitive for measuring the quality characteristics of frozen pink salmon (*Oncorhynchus gorbuscha*), chum salmon (*Oncorhynchus keta*), sockeye salmon (*Oncorhynchus nerka*).

1. Introduction

One of the fundamental tasks of the Fisheries Industries is to ensure the preservation of the high-quality fishery products, the manufacturing of products with the predetermined customer properties and the guaranteed shelf life.

In order to comply with these fundamental task prerequisites, it is obligatory to be familiar with the processes occurring during the fish storage and processing, to know how to manage them and to have an opportunity to control the basic indexes characterizing its quality.

During fishing auctions in the European Community countries, the sensory quality of fish (fish quality index) is used for the evaluation of the freshness grade of the fishes. This quality index can also be used at fish unloading points, for calculation of the shelf life and for the storage conditions which are used for sorting before further processing [1]. Therefore, the sensory properties such as: taste, sight, smell, and touch are evaluated for the appearance, flavor, mucus condition, eyes, gills and abdominal cavity of the whole or gutted fish. On the basis of these indexes, the fish is referred to E (Extra) grade — that is the highest freshness grade, A, B or C grades (runoff product).

The rapid test quality index method (QIM) is used for the chilled fish [2]. The present method is based on consideration of the specific changes in the fish, namely appearance, flavor and consistence changes. The penalty points from 0 to 3 are calculated for each index, then the points are summarized by all the indexes and the total sensory estimate is obtained, that is known as the “quality index”, linearly increasing with the fish storage time. Under modern conditions the method mentioned above allows for quick and objective evaluation of the fish quality by means of the special program, adapted for different computer-based systems. Evaluation can be performed at any stage: during the fishery products delivery at production site, fish storage or sale. The disadvantage of QIM is that it is applied only to the proven testing groups and it can be used only for whole fish.

It is well known that autolysis and bacterial deterioration lead to changes in concentrations of adenosine-5'-triphosphate [ATP], adenosine-5'-diphosphate [ADP], adenosine-5'-monophosphate [AMP] and inosine-5'-monophosphate [IMP], which convert quantitatively in inosine [Ino] and hypoxanthine [Hx]. It has been reported that there was a good correlation relating the decrease of fish freshness with the increase in nucleotides formation for a large number of fish species [3,4,5]. In this context the quality factor K was used for the quantitative evaluation

of fish freshness. The quality factor K is defined by the following equation [6,7,8]:

$$K = \frac{\text{Ino} + \text{Hx}}{\text{ATP} + \text{ADP} + \text{AMP} + \text{IMP} + \text{Ino} + \text{Hx}} * 100, \% \quad (1)$$

It has been suggested that the determination of adenosine-5'-triphosphate degradation products content in the fish can be used as the basis of the quality factor K calculation, when evaluating post-mortem fish quality changes during fish storage [8].

As a result, it became considerably interesting not only to analyze the content of metabolites in the chilled fish during their storage but also to characterize the raw fish quality, from which the frozen product was manufactured.

According to Karube [9] for some fish species ATP decomposes very quickly to IMP and the definition of quality factor K can be modified by excluding the adenosine phosphates and suggested a new calculation quality factor K_1 defined as:

$$K_1 = \frac{\text{Ino} + \text{Hx}}{\text{IMP} + \text{Ino} + \text{Hx}} * 100, \% \quad (2)$$

Luong [10] proved that, for some species, quality factor K and K_1 do not adequately reflect the alterations and proposed the quality factor H based on Hx concentration to be considered as a good indicator of fish freshness under physiological and sensory points of view:

$$H = \frac{\text{Hx}}{\text{IMP} + \text{Ino} + \text{Hx}} * 100, \% \quad (3)$$

In recent years, it has been shown that NMR spectroscopy is a good tool for assessing the quality of fish raw materials [7,8]. The fish quality factor can be calculated by analyzing the NMR spectra and determining the concentration of ATP decay products.

In the work, NMR spectroscopy was used to assess the quality of salmon species of fish. The research was conducted on chilled Atlantic salmon (*Salmo Salar*) as the most massive fish, sold through retail chains as chilled and frozen pink salmon (*Oncorhynchus gorbuscha*), chum salmon (*Oncorhynchus keta*), sockeye salmon (*Oncorhynchus nerka*) which are used in trade and processing as frozen.

2. Materials and methods

2.1. Sample preparation

The chilled Atlantic Salmon (*Salmo Salar*) with storage duration of 9 days produced on the Faroe Islands (starting from the preparation date) and on the 18 day of storage (from the prepara-

tion date) at the temperature of approximately 0 °C, frozen pinc salmon (*Oncorhynchus gorbuscha*), chum salmon (*Oncorhynchus keta*), sockeye salmon (*Oncorhynchus nerka*) with storage duration about three months at the temperature of approximately minus 18 °C were used as objects for research. For the analysis were used average muscular samples of different parts, of several chilled and frozen fish.

The protein content was determined on the autoanalyzer «Kjeltec» Foss-2300 (Sweden) according to the Kjeldahl method [11]. Moisture content was determined in according to GOST 7636 [11]. Lipids content was defined on the automated extractor SER148/6 manufactured by VELP company according to the Soxhlet method. The content of Total Volatile Basic Nitrogen (TVB-N) was determined on the semi-automatic distillation unit K-355 (Buchi company, Switzerland).

2.2. Sample preparation for NMR-spectroscopy

The water soluble polar metabolites of fish samples were extracted by 7.5% solution of trichloroacetic acid (TCA), as it was described in the paper [7]. For this purpose 25 g of fish muscle was added to 50 mL of 7.5% TCA and homogenized using a vertical homogenizer. The homogenate was filtered through the paper filter. The filtrate was neutralized by 9 M solution of KOH up to pH value of 7.8. The solution was filtered through the paper filter (№ 1) and it was stored at the temperature minus 40 °C until the measurements conduction.

2.3. NMR-spectroscopy performance

For NMR-spectral data collection the samples were thawed and dissolved in D₂O. The NMR-spectral data was collected with Bruker AV-600 spectrometer (Germany) at 30 °C with TSP (3-(trimethylsilyl)-propionic-2,2,3,3-*d*₄ acid sodium salt) taken as the internal standard (δ_H 0.0, δ_C -1.6). All metabolites marked at spectra were identified with the use of 1D (¹H, ¹³C) and 2D (¹H, ¹H COSY, TOCSY, ROESY и ¹H, ¹³C HSQC, HMBC) NMR-spectroscopy, and also on the basis of literature values [7,8,12]. Spectra processing was performed with the use of standard software package of the Bruker company (TopSpin 3.6.1)

3. Results and discussion

3.1. Proximate analysis

Proximate analysis chemical composition of the fish muscle of Atlantic salmon (*Salmo Salar*), pink salmon (*Oncorhynchus gorbuscha*), chum salmon (*Oncorhynchus keta*), sockeye salmon (*Oncorhynchus nerka*) are presented in Table 1.

Table 1

Chemical composition of the fish muscle

Title	Content, %			
	moisture	lipids	ashes	protein
Atlantic Salmon (<i>Salmo Salar</i>)	60.20±0.07	17.70±0.10	1.90±0.12	20.20±0.10
Pinc salmon (<i>Oncorhynchus gorbuscha</i>)	71.46±0.05	5.32±0.02	1.71±0.10	21.51±0.11
Chum salmon (<i>Oncorhynchus keta</i>)	73.70±0.07	4.08±0.02	1.55±0.08	20.67±0.08
Sockeye salmon (<i>Oncorhynchus nerka</i>)	69.40±0.05	7.32±0.02	1.67±0.11	21.61±0.10

The quality estimation of the fish samples studied were obtained from different conditions of storage (duration) and the sensory quality of fish were estimated and TVB-N values determined (see the results in Table 2).

As it is seen from the presented data on 18 day of storage the chilled fish had the off-odor and changed the color.

The TVB-N index was 31 g/100 g. According to Commission Regulation EC No 1022/2008/EC of 17 Dec. 2008, which makes amendments in Regulation EC № 2074/2005 in respect of the critical concentrations of Total Volatile Basic Nitrogen (TVB-N) [13] the critical content of TVB-N should be 35 mg/100 g for salmon fishes. When the present value exceeds 35 mg/100 g, the product is considered inedible and unsuitable for industrial processing.

Table 2

Sensory quality of fish muscle and TVB-N content

Title	Organoleptic muscular indicators	Storage duration, days	Storage temperature, °C	TVB-N content, mg/100 g
Atlantic Salmon (<i>Salmo Salar</i>)	Bright color, no evidence of oxidation, no off-odor	9	0	22.4
Atlantic Salmon (<i>Salmo Salar</i>)	Color with shade of orange, showing signs of oxidation, the off-odor of rank fish	18	0	31.1
Atlantic Salmon (<i>Salmo Salar</i>)	After thawing the color is bright, no evidence of oxidation, no off-odor	9	minus 20	24.1
Pinc salmon (<i>Oncorhynchus gorbuscha</i>)	After thawing the color is characteristic of the species, there is no evidence of oxidation and no off-odor	90	minus 18	14.0
Chum salmon (<i>Oncorhynchus keta</i>)	After thawing the color is characteristic of the species, there is no evidence of oxidation and no off-odor	95	minus 18	9.8
Sockeye salmon (<i>Oncorhynchus nerka</i>)	After thawing the color is bright red, there is no evidence of oxidation and no off-odor	98	minus 18	12.6

According to the TVB-N index chilled Atlantic Salmon with storage duration of 18 days at 0 °C met the requirements applicable to food fishery products suitable for industrial processing and direct consumption. But at the same time on the basis of the sensory quality of fish it could not be recommended for direct consumption. Consequently, the TVB-N index which is recommended to be determined in case of discrepancies during evaluation of the sensory quality of the chilled or frozen fishery products, sometimes characterizes the product quality incorrectly.

3.2. NMR-spectra

The ¹H-NMR spectra of chilled Atlantic salmon (Salmon Salar) with 9 and 18 days storage duration at 0 °C muscle TCA extract is presented in Figure 1. The similar ¹H-NMR spectra were recorded for all fish samples.

The low-field region ¹H-NMR spectra of Pinc salmon (*Oncorhynchus gorbuscha*), Chum salmon (*Oncorhynchus keta*), Sockeye salmon (*Oncorhynchus nerka*) muscle TCA extract are presented in Figure 2. The signals of the following metabolites were assigned: lactic acid (LA), anserin (Ans), creatine (Crt), trimethylamine (TMA), choline (Cho), trimethylamine N-oxide (TMAO), adenosine-5'-triphosphate (ATP), adenosine-5'-diphosphate (ADP), adenosine-5'-monophosphate (AMP), inosine (Ino), hypoxanthine (Hx) and inosine-5'-monophosphate (IMP). The structural formulas and chemical shifts of ¹H and ¹³C of the metabolites mentioned above are presented in Table 3.

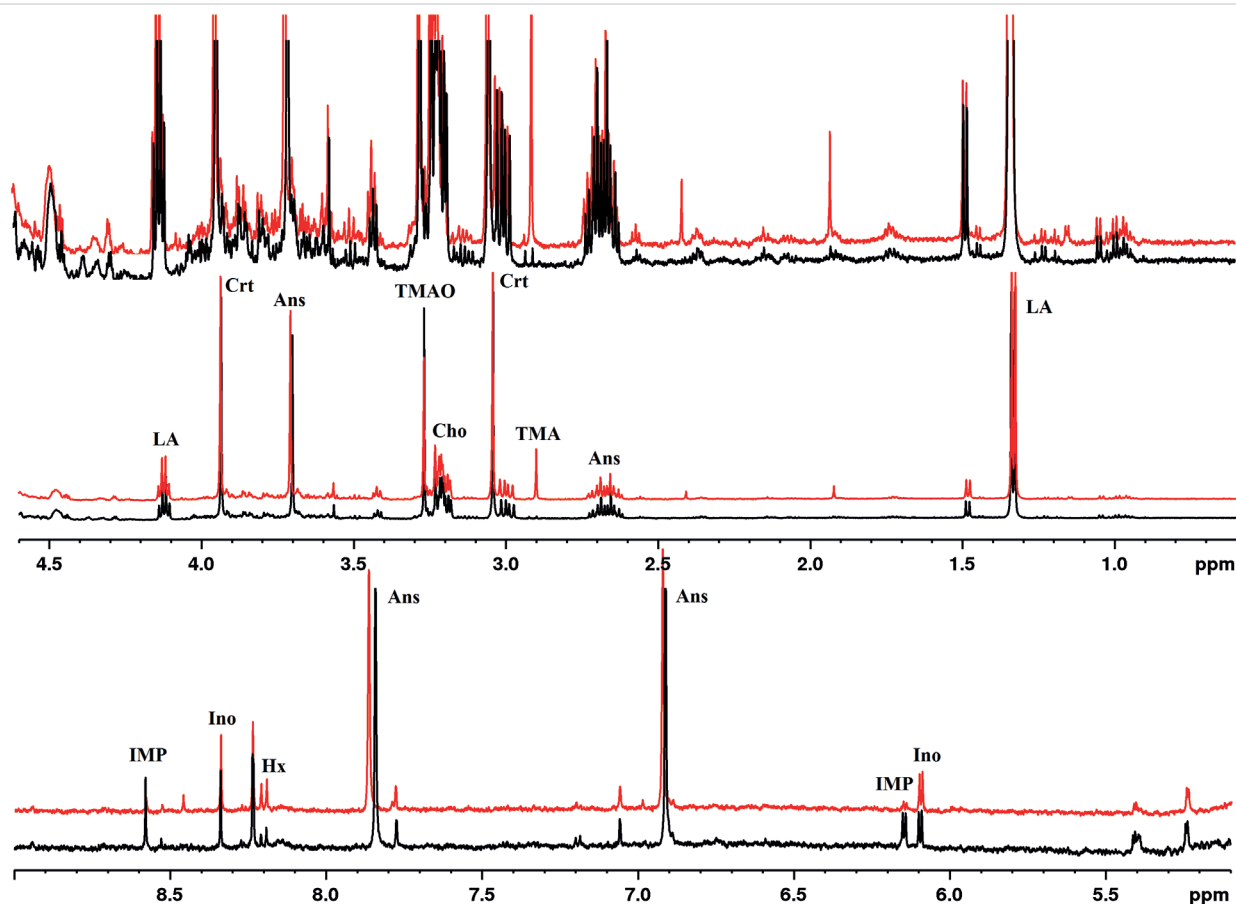


Figure 1. ^1H -NMR spectrum of chilled Atlantic Salmon (*Salmo Salar*) muscle TCA extract with 9 days (black color) and 18 days (red color) storage duration at 0 °C

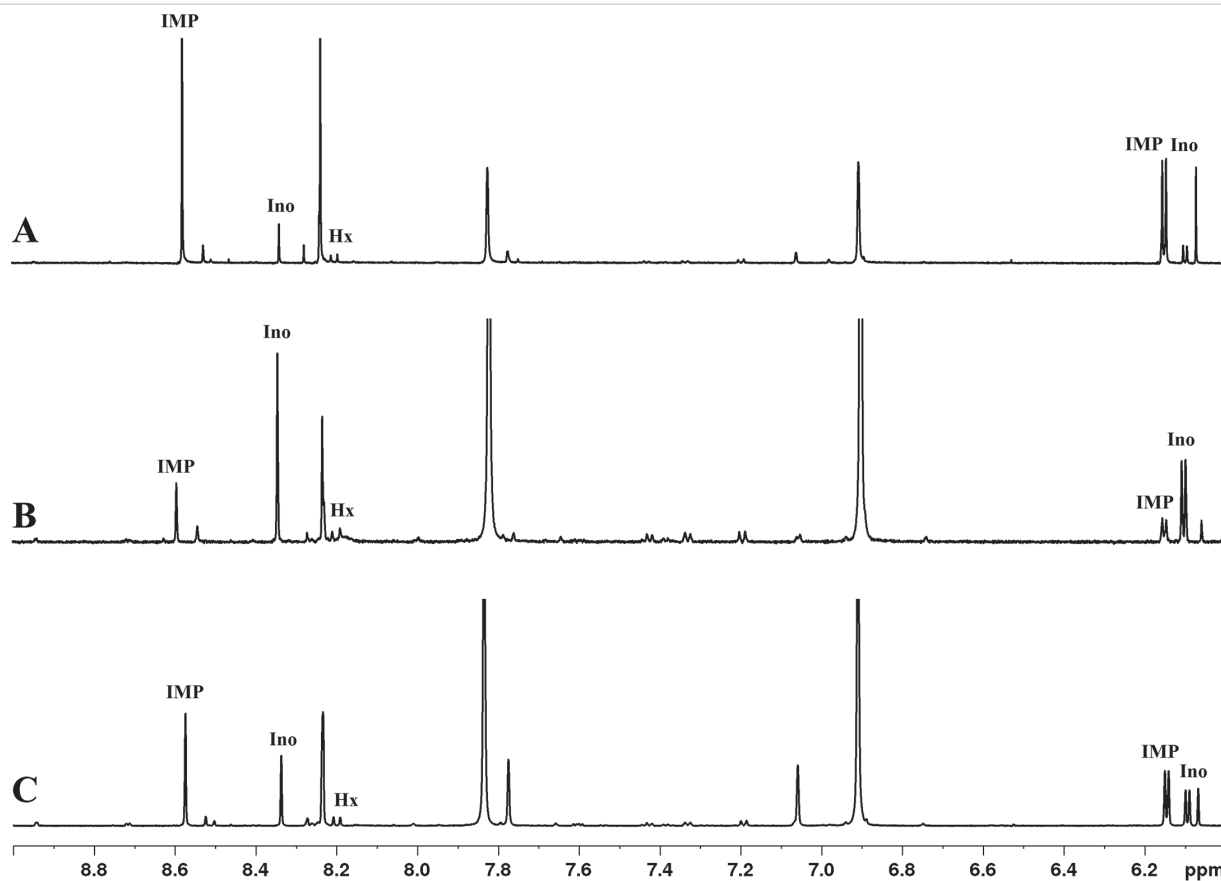
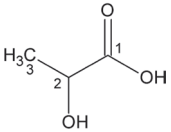
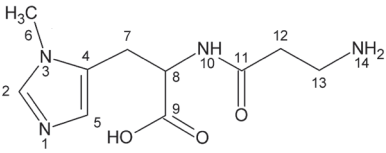
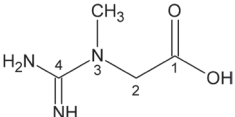
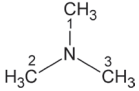
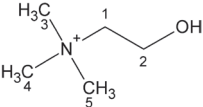
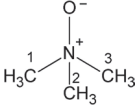
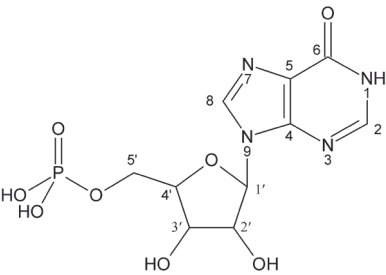
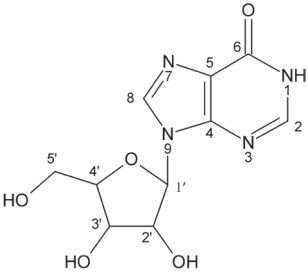
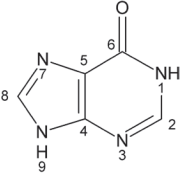


Figure 2. The low-field region ^1H -NMR spectra of fish muscle TCA extract: A) Pinc salmon (*Oncorhynchus gorbuscha*), B) Chum salmon (*Oncorhynchus keta*), C) Sockeye salmon (*Oncorhynchus nerka*)

Table 3

Chemical shifts of ^1H and ^{13}C of the metabolites identified in $^1\text{H} - ^{13}\text{C}$ HSQC NMR spectra of Salmon fish species muscle TCA extract

Metabolite	Chemical Structures	Carbon atom	Chemical shifts		
			proton δ_{H} , ppm	Multiplicity*	Carbon atom δ_{C} , ppm
Lactic acid (LA)		1			183.7
		2	4.12	d	69.7
		3	1.33	d	21.5
Ancerin (Ans)		2	7.84	s	138.2
		4			131.3
		5	6.91	s	123.1
		6	3.77	s	33.6
		7	3.22, 3.06	dd	27.3
		8	4.47	dd	54.8
		9			178.1
		11			172.8
		12	2.71	m	33.3
		13	3.26	dd	37.0
Creatine (Crt)		1			175.6
		2	3.94	s	55.2
		4			158.2
		5	3.06	s	38.4
Trimethylamine (TMA)		1,2,3	2.90	s	47.3
Choline (Cho)		3,4,5	3.24	s	54.8
Trimethylamine N-oxide (TMAO)		1,2,3	3.27	s	62.2
Inosin-5'-phosphate (IMP)		2	8.34		147.5
		4			149.5
		5			125.7
		6			160.0
		8	8.58	s	141.2
		1'	6.14	s	89.1
		2'	4.70	m	76.2
		3'	4.49	dd	71.5
		4'	4.36	m	85.5
		5'	4.06, 4.01	m	64.8
Inosine (Ino)		2	8.34	s	147.5
		4			149.5
		5			125.7
		6			160.0
		8	8.24	s	141.5
		1'	6.09	d	89.9
		2'	4.77	s	75.3
		3'	4.45	dd	71.5
		4'	4.29	dd	86.7
		5'	3.95, 3.86	dd	62.6
Hypoxantin (Hx)		2	8.21	s	146.7
		4			153.9
		5			120.7
		6			158.6
		8	8.19	s	143.1

* s — singlet; d — doublet; dd — doublet of doublet; m — multiplet.

3.3. Determination of adenosine-5'-triphosphate degradation products' content in fishery products

The analysis of ¹H-NMR weak field spectra has allowed us to establish that all the samples exhibited the absence of adenosine-5'-triphosphate, adenosine-5'-diphosphate and adenosine-5'-monophosphate signals, whereas they were characterized by the diagnostic signals of inosine, hypoxanthine and inosine-5'-monophosphate.

This obtained data was in good agreement with the results published in the literature [14,15]. Indeed, these authors established that after post-mortem changes, the total decomposition of ATP occurred and the analyzed fish contained metabolites such as inosine-5'-monophosphate, inosine and hypoxanthine. Therefore, in our manuscript the quality estimation of fish, relied on the identification of three metabolites: inosine-5'-monophosphate, inosine and hypoxanthine.

On the basis of ¹H-NMR spectra analysis, it is concluded that the value of the quality factor K for stored chilled and frozen raw materials can be calculated by the formula 2. The results are presented in Table 4.

It follows from the presented data that the chilled Atlantic salmon which were kept in storage for a duration of 18 days at 0°C gave unsatisfactory sensory quality (signs of oxidation and off-odor of rank fish) that had quality factor K₁ equal to 90%. This quality factor K₁, which defines the fish quality, was indicative for the biochemical changes occurring in fish due to their storing conditions. Consequently, the higher the biochemical changes, and consequently the higher value of quality factor K₁, indicate about loss of fish quality. This correlates well with degradation of the sensory quality of fish and the test of spoiled fish. The quality factor K₁ of more than 80% is the threshold quality value, when the product is considered to be unfit for food.

It is important to point out that quality factor K₁ for frozen samples of fish estimated under given above formula was too

high, which did not correlate with satisfactory sensory quality of fish and the TVB-N indexes for these samples. For these reasons and as proposed by others [10,12,14] the quality factor H was used for characteristics of frozen fish, estimated under the formula 3.

Obtained results in table 4 show that quality factor H more objectively reflects quality of frozen samples. It can be concluded that Pinc salmon (*Oncorhynchus gorbuscha*), chum salmon (*Oncorhynchus keta*), sockeye salmon (*Oncorhynchus nerka*) were frozen immediately after catching, as they have high sensory quality of fish and consequently low of quality factor H.

It allows to conclude that the following methodical approach can be used for characteristics of not only frozen fish but also for raw fish from which frozen pinc salmon (*Oncorhynchus gorbuscha*), chum salmon (*Oncorhynchus keta*), sockeye salmon (*Oncorhynchus nerka*) were produced. Thus the NMR-spectroscopy allows for objective evaluation of the fish chemical quality. Consequently, reliable information concerning the product can be obtained, the shelf life can be predicted and the tailor-made products manufacturing processes management can be performed on the basis of the comprehensive research and correlation with the organoleptic analysis as well as the nutritional quality and the processing properties evaluation.

4. Conclusion

It has been established that the fish quality index can be expressed by the quality factor K₁ which is calculated by means of analysis of ¹H-NMR spectra and concentration measurement of inosine, hypoxanthine and inosine-5'-monophosphate generated during the fish storage. The quality factor K₁ correlates well with the sensory quality of chilled fish. For quality estimation of frozen pinc salmon (*Oncorhynchus gorbuscha*), chum salmon (*Oncorhynchus keta*), sockeye salmon (*Oncorhynchus nerka*) quality factor H is more informative, which correlate with low indicators of TVB-N and high sensory quality of fish.

Table 4

Calculation quality factors K₁ and H for Salmon fish species samples of different storage duration

Title	Storage duration, days	Hypoxanthine Hx (8.21 ppm), a.u.*	Inosine Ino (8.34 ppm), a.u.	Inosine-5'-monophosphate IMP (8.58 ppm) a.u.	K ₁ , %	H, %
Atlantic Salmon (<i>Salmo Salar</i>)	9 0°C	0.334	1.453	1.326	57.4	10.7
Atlantic Salmon (<i>Salmo Salar</i>)	18 0°C	0.773	1.753	0.270	90.3	27.7
Atlantic Salmon (<i>Salmo Salar</i>)	9 -20°C	0.389	1.526	1.312	59.3	12.0
Pinc salmon (<i>Oncorhynchus gorbuscha</i>)	90 -18°C	0.436	1.208	8.277	16.6	4.4
Chum salmon (<i>Oncorhynchus keta</i>)	95 -18°C	0.420	3.313	1.041	78.2	8.8
Sockeye salmon (<i>Oncorhynchus nerka</i>)	98 -18°C	0.234	1.732	2.667	42.4	5.0

* a.u. — unit of area

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