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Hydrolysis products from sockeye (*Oncorhynchus nerka* L.) heads from the Kamchatka Peninsula produced by different methods: biological value

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Abstract:
Introduction. Sockeye salmon (*Oncorhynchus nerka* L.) is a valuable Pacific salmon. Sockeye heads are a significant share in processing sockeye salmon. Traditionally, fish by-products are used to make fishmeal. However, due to the high content of collagen proteins and fat in sockeye salmon heads, it is difficult to produce fishmeal from this raw material. Controlled enzymatic or combined hydrolysis allows protein, fat, and minerals to be extracted to supply the market with higher value products with desirable features. This research was aimed to analyze the chemical composition and biological value of hydrolysis products obtained from sockeye heads.
Study objects and methods. We investigated hydrolysis products of sockeye salmon heads, namely protein hydrolysates, fat and sludge. Thermal hydrolysis and enzymatic-thermal hydrolysis were used for the tests. Thermal hydrolysis was realized in reactor. For enzymatic-thermal hydrolysis, the raw material was pre-treated by proteolytic enzyme Alcalase. The hydrolysates obtained were investigated. Chemical composition was determined in accordance with State Standard 7636-85. HPLC was used for molecular weight and amino acid analysis. Gas chromatography was used for fatty acid analysis. Biological value of proteins was determined by the balance of the amino acid composition comparing it with the "ideal protein model".
Results and discussion. Thermal hydrolysis resulted in the production of protein hydrolysate powder with protein content of 92.0% dry matter and a protein recovery rate of 39.6%. Combined hydrolysis resulted in the production of protein hydrolysate powder with protein content of 92.6% and a protein recovery rate of 83%. All protein hydrolysates contained all essential amino acids. The biological value of protein hydrolysate obtained by thermal and combined hydrolysis was 80.1 and 82.8%, respectively.
Conclusion. Hydrolysed products obtained by thermal and enzymatic-thermal hydrolysis had a valuable chemical composition and could be recommended for food and feed use.

Keywords: Fish, by-products, sockeye, hydrolysis, amino-acid profile, peptides, protein

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INTRODUCTION

The highest actual salmon harvest – 676 thousand tons – was recorded last in 2018 [1]. In recent years, the average annual salmon harvest has been 280 thousand tons [2]. The total harvest of sockeye salmon of the Kamchatka Peninsula in 2018 was 41.1 thousand tons [3].

The average total annual sockeye salmon harvest worldwide is 140–180 thousand tons [4]. Sockeye salmon (*Oncorhynchus nerka* L.) is a valuable Pacific salmon. It is about 60 cm long with an average weight of 2 to 4 kg. In the Russian Far East, when processing sockeye salmon, by-products (heads, spines, etc.) account

for about 25% of the total weight, which is about 10 thousand tons a year [5]. By-products have a valuable chemical composition, but only about 20–30% of the total amount is used to produce fishmeal.

One of the hardest parts to process is the sockeye salmon head, which, on average, accounts for 14.8–17.9% of the total fish weight and 67–69% of the total waste amount. Sockeye salmon heads contain a high amount of collagen proteins (26–38% of the total mass of proteins), fat (15–18% of the total weight), and mineral substances (3.8–5.1% of the total weight) [6]. When cooking such raw materials, a colloidal emulsion mass with increased viscosity forms, which is hard to separate and dry. Therefore, final products do not meet the standard requirements for fishmeal, including in terms of physical characteristics, water and fat contents, while fat is rapidly oxidized, which leads to intoxication and product damage [7]. These semi-finished products cannot be used as food.

To use the valuable biological potential of sockeye salmon heads, hydrolysis methods were proposed that make it possible to separate fractions from organic raw materials – proteins and peptides, fats and fat-soluble compounds, mineral compounds with water insoluble protein components [8]. Mild hydrolysis makes it possible to preserve the valuable chemical composition of each fraction, and hydrolysis products can be recommended even for food purposes. Chemical hydrolysis (acidic or alkaline) damages certain essential amino acids, so waste water purification is necessary because of the high concentration of chemical components [9].

This research proposed and investigated two different hydrolysis, thermal and enzymatic-thermal. These two methods allow for deep hydrolysis of the complex protein-lipid-mineral system of sockeye salmon heads with maximum preservation of valuable amino acids and unsaturated fatty acids. Thermal hydrolysis is based on using high temperatures and pressure in an aqueous medium. Enzymatic-thermal hydrolysis includes pre-treatment of raw material with proteolytic enzymes followed by thermal hydrolysis [10]. Depending on the hydrolysis method and its parameters, it is possible to produce protein products with a different amino acid composition and molecular weight, fatty products with different fractional lipid composition, as well as protein-mineral or mineral-protein by-products from sludge with different biological values. The content of these biologically active substances predetermines the uses of the final products of hydrolysis.

STUDY OBJECTS AND METHODS

Frozen sockeye (*Oncorhynchus nerka* L.) heads were provided by LSC Ozernovskiy fish cannery factory № 55 (the Kamchatka region, Russia) in September 2016. In plastic boxes, they were delivered by plane within 24 h to the laboratory and then stored in a freezer at –18°C for one week until tests. Sockeye was caught in the southwestern part of the Kamchatka Peninsula, in one of the largest spawning grounds of sockeye salmon in the world [11].

Chemical composition of sockeye heads was determined in accordance with State Standard 7636-85[1]. The mass fraction of fat was determined in previously dried samples by extraction with diethyl ether according to the Soxhlet method. Nitrogen content was determined by the Kjeldahl method using a UDK 127 analyzer (VELP Scientifica, Italy) with pre-burning of the samples in sulfuric acid in the presence of hydrogen peroxide and mineral catalyst.

Thermal and enzymatic-thermal hydrolysis. Hydrolysis tests were carried out at the technology company ANiMOX (Adlershof, Berlin, Germany). Defrosted sockeye heads were minced and subjected to thermal and enzymatic-thermal hydrolysis. Hydrolyzed mixture was cooled down and separated by centrifugation into three fractions: protein, fat and sludge (protein-mineral or mineral-protein by-products). The aqueous fraction (water-soluble proteins) was freeze-dried to produce a protein hydrolysate. The sludge was subjected to convectional drying to obtain a protein-mineral or mineral-protein product.

Thermal hydrolysis (T-hydrolysis) of minced raw materials was realized in a reactor for 60 min at 130°C and pressure 0.20 MPa (pH 7.0). After hydrolysis, the organic mixture was separated using a centrifuge at 3500 rpm for 10 min.

During enzymatic-thermal hydrolysis (ET-hydrolysis), the raw materials were at first treated by proteolytic enzyme Alcalase 2.5 L (Novozymes) for 6 h at 50°C and 130 rpm (pH 8.0). Minced raw material was mixed with hot water at a 1:1 ratio. Subsequent fractionation was carried out by centrifugation with parameters described above for T-hydrolysis.

The chosen hydrolysis parameters showed good results with relatively fatty sardine cannery by-products in our previous investigations [10]. We applied T-hydrolysis to produce simultaneously basic protein-mineral fish meal and high value added fish protein hydrolysate with mostly medium molecular weight of proteins between 10 kDa and 100 kDa. ET-hydrolysis was used to produce high value added fish protein hydrolysate with mostly low molecular weight of proteins under 10 kDa.

Molecular weight distribution. The molecular weight profile of protein hydrolysates was estimated by the SEC method on a Merck/Hitachi HPLC system (detection UV 213 nm) with a Phenomenex Yarra SEC-2000 column (300×7.8 mm). Degassed 0.1 M potassium phosphate buffer with pH of 6.8 was used as a mobile phase. Ten microliters of the previously prepared material with 0.45-micron pore size filtered

[1] State Standard 7636-85. Fish, marine mammals, invertebrates and products of their processing. Methods of analysis. Moscow: Standartinform; 2010. 86 p.

Table 1 Chemical composition of sockeye heads

Material	Dry matter, %	Protein (% of dry matter)	Fat (% of dry matter)	Ash (% of dry matter)
Sockeye heads	34.1	12.5 (36.7)	16.9 (49.6)	4.47 (13.1)

sample at a concentration of 0.2–0.3% dry matter was used for each measurement. Calibration was carried out in triplicate using the BIO-RAD Gel Filtration Standard 151-1901. The mean retention time and molecular weight were plotted on a half-logarithmic scale. The linear correlation was used to interpolate the molecular weight ranges under analysis. Accordingly, the sample chromatograms were integrated piecewise between the retention time estimated.

Amino-acid profile. The amino acid profile of proteins was determined at the UBF laboratory (Altlandsberg, Germany) using HPLC after hydrolysis of proteins in 6 N boiling hydrochloric acid for 48 h. O-phthalaldehyde was used for derivatization. HPLC detection was performed using Agilent 1200 Series G1379A for UV-detection, G1312A for fluorescence detection, and G1329A for diode-array detection.

Fatty acid composition. The fatty acid composition of sockeye head hydrolysis products was analyzed at the UBF laboratory (Altlandsberg, Germany) by gas chromatography. Transesterification was carried out by DGF standard procedure using TMSH (trimethylsulfonium hydroxide) in tert-butyl-methylether. Quantification was done by reference standard mixtures (Supelco, Merck). An analytical system was GC 2010 from Shimadzu (Kyoto, Japan) equipped with flame ionization detection and computer system. The gas chromatography values were quantified by response corrected total area principal. Phase separation was performed using SP2380 (Supelco), 0.2 µm film thickness, 0.5 mm diameter, 25 m. Detector: 250°C (temperature program starting at 75°C with 5 K/min to 125°C, followed by 2 K/min to 225°C). Equilibration time was 5 min.

Table 2 Chemical composition of raw materials and fractions obtained after thermal hydrolysis of sockeye heads

Sample	Dry matter, kg/100 kg	Protein, kg/100 kg	Fat, kg/100 kg	Ash, kg/100 kg	Share of fraction, %
Sockeye heads and water	17.0	6.27 (37.0)	8.46 (49.9)	2.24 (13.2)	100
Protein fraction	3.89	3.58 (92.0)	0.03 (0.69)	0.29 (7.34)	69.5
Sludge (protein-mineral fraction)	29.9	16.3 (54.7)	4.74 (15.9)	8.80 (29.4)	23.2
Fat fraction	100	nd	nd	nd	7.34

Values in brackets are used in relation to the dry matter

nd = not detected

Table 3 Chemical composition of raw materials and fractions obtained after enzymatic-thermal hydrolysis of sockeye heads

Sample	Dry matter, kg/100 kg	Protein, kg/100 kg	Fat, kg/100 kg	Ash, kg/100 kg	Share of fraction, %
Sockeye heads and water	17.0	6.27 (37.0)	8.46 (49.9)	2.24 (13.2)	100
Protein fraction	6.71	6.21 (92.5)	0.07 (1.01)	0.43 (6.47)	83.8
Sludge (mineral-protein fraction)	44.7	12.3 (27.4)	10.9 (24.5)	21.5 (48.1)	8.70
Fat fraction	100	nd	nd	nd	7.45

Values in brackets are used in relation to the dry matter, kg/100 kg

nd = not detected

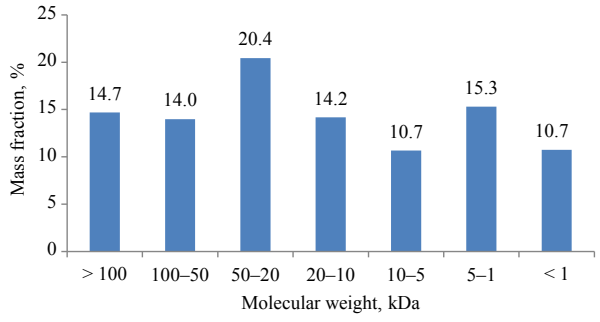

Figure 1 Protein distribution based on molecular weight of peptides and their share in protein hydrolysates from sockeye heads after thermal hydrolysis

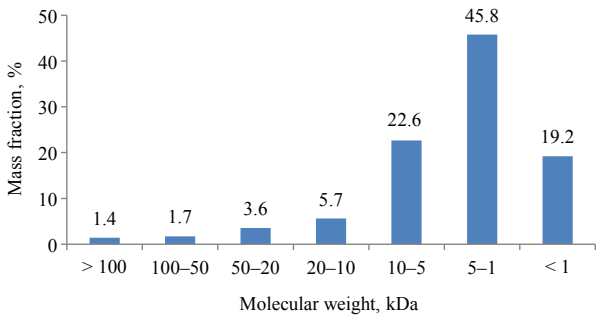

Figure 2 Protein distribution based on molecular weight of peptides and their share in protein hydrolysates from sockeye heads after thermo-enzymatic hydrolysis

Biological value. The biological value of proteins was determined by the balance of the amino acid composition by comparing it with the "ideal protein model" [12]. Amino-acid score was calculated according to the methodology of FAO/WHO using Eg. (1). The excessive amount of essential amino acids was calculated according to the formula of the coefficient of amino acid score difference (CASD) (Eg. (2)). Based on CASD biological value of proteins was calculated (Eq. (3)).

$$AS_i = \frac{AA_i}{AA_{i\,st}} \cdot 100 \quad (1)$$

where AS_i is the amino-acid score of the i-th essential amino acid, %; AA_i is the content of the i-th essential amino acid in 100 g of the analyzed protein, g; and is the content of the same essential amino acid in 100 g of the standard ("ideal") protein, g.

$$CASD = \frac{\sum \Delta DAS}{n} \quad (2)$$

where CASD is the coefficient of amino-acid score difference, %; is the difference of the amino-acid score of the i-th essential amino acid, %; AS_{min} is the minimal score for an essential amino acid in the analyzed protein, %; and *n* is the number of essential amino acids in the analyzed protein.

$$BV = 100 - CASD \quad (3)$$

where BV is biological value of the analyzed protein, %.

RESULTS AND DISCUSSION

Table 1 represents the chemical composition of sockeye heads (*Oncorhynchus nerka* L.).

Sockeye heads are a valuable raw material with great amounts of useful components.

Chemical composition of protein hydrolysates from sockeye heads after thermal hydrolysis. After thermal hydrolysis and separation of the organic suspension by centrifugation, three fractions were

Table 4 Amino-acid profile of lyophilized protein hydrolysates from sockeye heads produced by different hydrolysis methods

Indicators	T-hydrolysis		ET-hydrolysis		"Ideal" protein by FAO/WHO, g/100 g
	Content, g/100 g	Amino-acid score, %	Content, g/100 g	Amino-acid score, %	
Non-essential amino acids					
Alanine	6.68		5.51		
Arginine	7.01		4.79		
Asparagine	0		0.01		
Aspartic acid	5.08		5.27		
Citrullin	0.02		0.03		
Cystine	0.11		0.22		
Glutamine	0.02		0.02		
Glutamic acid	10.97		8.16		
Glycine	17.38		9.74		
Histidine	1.48	98.7	1.54	102.7	
Hydroxyproline	4.74		2.31		
Ornithine	0.10		0.11		
Proline	6.21		4.88		
Serine	3.35		3.98		
Taurine	2.40		1.20		
Tyrosine	0.85		1.49		
Essential amino acids					
Isoleucine	2.06	49.1	2.72	64.8	4.2
Leucine	2.84	59.2	4.49	93.5	4.8
Lysine	4.38	104.3	4.82	114.8	4.2
Methionine	2.24	77.2	2.06	71.0	2.9
Phenylalanine	2.44	87.1	2.21	78.9	2.8
Threonine	2.63	93.9	2.49	88.9	2.8
Tryptophan	n/a	n/a	n/a	n/a	n/a
Valine	no data	no data	2.79	66.4	4.2
Biological value of protein, %		80.13		82.79	
Protein content, g/100 g	90.2		90.7		
Dry matter, %	98.0		98.0		
Protein recovery rate, %	39.6		83.0		

Tryptophan is destroyed by the analysis method used therefore it was not taken into account in the calculation of amino-acid scores
n/a = not available

Table 5 Fractional composition of fats in protein hydrolysates from sockeye heads after thermal and enzymatic-thermal hydrolysis

Fractions of fatty acids	Fatty acid content, %	
	T-hydrolysis	ET-hydrolysis
Saturated fatty acids (SFAs)	29.3	32.7
Monounsaturated fatty acids (MUFAs)	39.2	43.7
Polyunsaturated fatty acids (PUFAs)	30.6	22.8
Trans fats	1.6	< 0.1
PUFAs:MUFAs:SFAs ratio	1:1.28:0.96	1:1.91:1.43
Mass fraction of fat,% of total mass of protein hydrolysate	0.68	0.99

Table 6 Fractional composition of fatty acids in fat from sockeye heads after thermal and enzymatic-thermal hydrolysis

Fractions of fatty acids	Fatty acid content, %	
	T-hydrolysis	ET-hydrolysis
Saturated fatty acids (SFAs)	22.3	23.2
Monounsaturated fatty acids (MUFAs)	54.8	42.9
Polyunsaturated fatty acids (PUFAs)	22.2	33.1
Trans fats	0.2	0.2
PUFAs:MUFAs:SFAs ratio	1:2.46:1	1:1.29:0.7
Mass fraction of fat,% of total mass of protein hydrolysate	86.8	88.1

produced, namely protein, fat, and sludge. Their chemical composition is presented in Table 2.

After thermal hydrolysis the protein fraction contained 92% of protein of dry matter. The fat was dry matter accounted for 100%. Rates of protein and fat extraction were 39.6 and 86.8%, respectively.

Molecular weight distribution of protein hydrolysates from sockeye heads after thermal hydrolysis. Figure 1 represents the distribution of proteins based on their molecular weight in protein hydrolysates after T-hydrolysis. The share of proteins with molecular weight over 20 kDa was 49.1%, while the share of proteins with molecular weight under 10 kDa (easily digestible peptides) was 36.7%. This shows that the major share of proteins after T-hydrolysis had a medium molecular weight.

Chemical composition of protein hydrolysates from sockeye heads after thermo-enzymatic hydrolysis. The chemical composition of fractions obtained by centrifugation of organic suspension after ET-hydrolysis is shown in Table 3.

After ET-hydrolysis, the protein fraction contained 92.5% of protein based on dry matter. The dry matter of fat accounted for 100%. Protein extraction rate was 83.0%, and the rate of fat extraction was 88.1%. ET-hydrolysis demonstrated a significantly increase in the extraction degree of biologically active protein and fat components from sockeye heads and a decrease in by-products compared to T-hydrolysis.

Molecular weight distribution in protein hydrolysates from sockeye heads after thermo-enzymatic hydrolysis. Figure 2 shows the distribution of proteins based on their molecular weight in protein hydrolysates after ET-hydrolysis. The share of proteins with molecular weight under 10 kDa (easily digestible peptides) was 87.6%. This implies that ET-hydrolysis allows producing protein hydrolysates with low molecular weight. This protein hydrolysate can be used as a highly digestible protein source for nutritional purposes.

Amino-acid profile of protein hydrolysates from sockeye heads. Table 4 shows amino acid profiles of freeze-dried protein hydrolysates from sockeye heads produced by different hydrolysis methods, as well as their biological value calculated based on amino acid balance using amino-acid score of essential amino acids and CASD (coefficient of amino acid score difference).

Table 7 Fatty acid composition of fat in sockeye heads after thermal- and enzymatic-thermal hydrolysis

Fatty acids	Fatty acid content, %	
	T-hydrolysis	ET-hydrolysis
Myristic	4.54	4.60
Pentadecylic	0.47	0.48
Palmitic	14.76	15.19
Palmitoleic	6.12	6.13
Margaric	0.73	0.77
Margaroleic	0.35	0.35
Stearic	2.09	2.28
Oleic	18.65	18.88
Vaccenic	3.85	3.85
Linoelaidic	0.23	0.23
Linoleic	1.88	1.94
Gamma-linolenic	0.93	0.92
Arachidic	0.18	0.18
Alpha-linolenic	3.80	4.30
Gondoic	12.52	12.26
Eicosadienoic	0.38	0.38
Eicosatrienoic	1.25	12.41
Behenic	0.24	0.32
Erucic	12.26	0.37
Docosadienoic	7.79	7.30
Arachidonic	0.14	0.00
Eicosapentaenoic	0.67	0.65
Lignoceric	0.00	0.17
Nervonic	1.08	1.11
Docosahexaenoic	5.11	4.96
TOTAL	100.00	100.00
Total SFAs	22.27	23.21
Total MUFAs	54.83	42.94
Total PUFAs	22.17	33.08
Total trans fatty acids	0.23	0.23
Total omega-3 fatty acids	10.82	22.32

According to Table 4, amino acid profiles of protein hydrolysates produced by T-hydrolysis and ET-hydrolysis were very similar. Hydrolysates contained all the essential amino acids and could be called high value proteins. The main amino acids were glutamic acid (8.16–10.97% of protein), glycine (9.74–17.38%), and proline (4.88–6.21%). They are characteristic for collagen proteins [13]. The assessment of the balance of proteins by amino-acid score and biological value in relation to the "ideal" protein showed that the biological value of protein hydrolysates after T-hydrolysis was 80.13%, and that of protein hydrolysates after ET-hydrolysis was 82.79%.

In comparison with the market product Amizate made in Norway out of salmon raw materials the protein hydrolysate have similar amino acid profile, especially after ET-hydrloysis [14]. This shows a high utilization potential of the Russian salmon fish by-products.

Fatty acid composition of fats in protein hydrolysates from sockeye heads. Table 5 describes the composition of fats (present in small amounts 0.68–0.99% of mass) in protein hydrolysates after T- and ET-hydrolysis. T-hydrolysis separated fat a bit better than ET-hydrolysis (0.68% vs. 0.99%). The proportion of polyunsaturated fatty acids (PUFAs) after T-hydrolysisis was larger than that after ET-hydrolysis (30.6% vs 22.8% of fat). The advantage of ET-hydrolysis is the minimum content of trans fat in the fat fraction (less than 0.1%).

Fatty acid composition of fat from sockeye heads after hydrolysis. Fractional analysis of fat from sockeye heads after T- and ET-hydrolysis (Table 6) showed that the yield after ET-hydrolysis was higher than after T-hydrolysis, and a PUFAs:MUFAs:SFAs ratio was more preferable after ET-hydrolysis. According to recommendations of FAO/WHO experts, this ratio is approximately equal to 0.6–1:1:1 [15].

Table 8 Amino-acid profiles of sediments after hydrolysis of sockeye heads after thermal and enzymatic-thermal hydrolysis

Indicators	T-hydrolysis		ET-hydrolysis		"Ideal" protein by FAO/WHO, g/100 g
	Content, g/100 g	Amino-acid score, %	Content, g/100 g	Amino-acid score, %	
Non-essential amino acids					
Alanine	3.56		2.34		
Arginine	3.97		1.95		
Asparagine	4.11		2.22		
Aspartic acid	0		0		
Citrullin	0.01		0		
Cystine	0.38		0		
Glutamine	0.01		0		
Glutamic acid	6.99		3.19		
Glycine	6.41		3		
Histidine	1.49	99.3	0.67	44.5	1.5
Hydroxyproline	1.16		0.77		
Ornithine	0.05		0.06		
Proline	2.26		1.65		
Serine	2.79		0.56		
Taurine	2.15		0.33		
Tyrosine	0		0.52		
Essential amino acids					
Isoleucine	2.69	64.0	1.48	35.2	4.2
Leucine	2.38	49.6	1.90	39.6	4.8
Lysine	4.30	102.4	1.94	46.2	4.2
Methionine	1.83	63.1	0.57	19.7	2.9
Phenylalanine	2.79	99.6	1.32	47.1	2.8
Threonine	0.55	19.6	1.08	38.6	2.8
Tryptophan	n.a.		n.a.		n/a
Valine	1.65	39.3	1.76	41.9	4.2
Biological value of protein, %		66.51		41.24	
Protein content, g/100 g	53.6		26.9		
Dry matter, %	98		98		

Tryptophan is destroyed by used analysis method therefore it was not taken into account in the calculation of amino-acid scores
n.a. = not available

Table 9 Fractional composition of fatty acids in by-products from sockeye heads after thermal and enzymatic-thermal hydrolysis

Fractions of fatty acids	Fatty acid content, % fat	
	T-hydrolysis	ET-hydrolysis
Saturated fatty acids (SFAs)	25.5	25.2
Monounsaturated fatty acids (MUFAs)	58.8	49.1
Polyunsaturated fatty acids (PUFAs)	15.0	24.9
Trans fats	0.1	< 0.1
PUFAs:MUFAs:SFAs ratio	1:3.92:1.7	1:1.97:1.01
Mass fraction of fat, % of total mass of protein hydrolysate	15.9	24.5

Sockeye fat samples after T- and ET-hydrolysis had a valuable fatty acid composition and could be recommended for food and feed use (Table 7). Both fat samples were rich in monounsaturated omega-9 oleic and gondoic fatty acids and most common saturated palmitic fatty acid. Fat after T-hydrolysis contained significantly more monounsaturated omega-9 erucic fatty acid. Fat after ET-hydrolysis contained a significantly higher amount of the rare polyunsaturated omega-3 eicosatrienoic essential fatty acid. Total amount of omega-3 fatty acids in fat after ET-hydrolysis was twice as large as that after T-hydrolysis (22.32% vs. 10.82%). These fatty acids have beneficial bioactivities including prevention of atherosclerosis, protection against maniac–depressive illness and various other medicinal properties [16].

Preliminary defatting of salmon by-products using relatively low temperature methods helps extracting fat raw materials [17]. This saves some enzyme costs and improve the quality of fat products as they are not processed using enzymes and high temperatures.

Amino-acid profile of the sludge from sockeye heads. As a result of hydrolysis, water-insoluble proteins and minerals formed a protein-mineral or mineral-protein sludge product. The yield of the protein fraction essentially depended on the type of hydrolysis [18]. T-hydrolysis produced much more protein than ET-hydrolysis (53.6% vs. 26.9%). Amino acid profiles of the sludge fractions were different (Table 8).

These by-products contained all the essential amino acids but their contents were lower than in protein hydrolysates including glutamic acid and glycine. The biological value of the protein-mineral product after T-hydrolysis was much higher than that one of mineral-protein product after ET-hydrolysis (66.51% vs. 41.24%). This can be explained by the fact that during ET-hydrolysis there is a deeper splitting of the protein chains and their transition into a soluble state is more intense therefore non-hydrolyzed proteins in the sludge contain less essential amino acids. The biological value of protein-mineral and mineral-protein products was much lower than that of protein hydrolysates.

Table 10 Fatty acid composition of fats in by-products from sockeye heads after thermal and enzymatic-thermal hydrolysis

Fatty acid	By-product T-hydrolysis, %	By-product ET-hydrolysis, %
Myristic	4.86	5.05
Pentadecylic	0.52	0.52
Palmitic	17.11	16.76
Palmitoleic	6.58	7.08
Margaric	0.79	0.78
Margaroleic	0.67	0.69
Stearic	2.58	2.37
Oleic	20.59	22.20
Vaccenic	3.98	4.18
Linoelaidic	0.15	0.00
Linoleic	1.69	1.42
Gamma-linolenic	0.65	0.50
Arachidic	0.18	0.17
Alpha-linolenic	4.53	5.39
Gondoic	13.00	13.75
Eicosadienoic	0.34	0.30
Eicosatrienoic	1.41	13.71
Behenic	0.23	0.31
Erucic	12.64	0.00
Docosadienoic	0.13	0.14
Arachidonic	3.54	1.84
Eicosapentaenoic	0.29	0.62
Lignoceric	0.00	0.00
Nervonic	1.32	1.22
Docosapentaenoic	0.00	0.00
Docosahexaenoic	2.23	1.02
TOTAL	100.00	100.00
Total SFAs	25.47	25.17
Total MUFAs	58.78	49.12
Total PUFAs	14.95	24.93
Total trans fatty acids	0.15	0.00
Total omega-3 fatty acids	8.46	20.73

Fatty acid composition of sludge from sockeye heads. Table 9 represents fractional composition of fatty acids in by-products after T- and ET-hydrolysis. These products contained a higher amount of fat after ET-hydrolysis (29.4%) compared to that after T-hydrolysis (15.9%).

By-products from sockeye heads obtained after T- and ET-hydrolysis were rich in monounsaturated omega-9 oleic and gondoic fatty acids and most common saturated palmitic fatty acid (Table 10). By-product after T-hydrolysis contained a significant amount of monounsaturated omega-9 erucic fatty acid. By-product after ET-hydrolysis contained no erucic fatty acid nut contained significantly higher amount of polyunsaturated omega-3 eicosatrienoic essential fatty acid. Total amount of omega-3 fatty acids in the by-

product after ET-hydrolysis is more than double of that after T-hydrolysis (20.73% vs 8.46%).

The data showed a difference in the content of polyunsaturated fatty acids and omega-3 fatty acids in fat and by-product fractions. In the by-products, this content was lower, especially of docosahexaenoic acid. This means that high-temperature treatment, sedimentation, and contact with mineral substances of the sedimentary part led to the fat quality deterioration but demonstrated pretty good fatty acid composition.

CONCLUSION

Processing sockeye (*Oncorhynchus nerka* L.) salmon heads through thermal and enzymatic-thermal hydrolysis resulted in the production of three products with valuable chemical composition, namely protein hydrolysates, fish oil, and sludge. Highly concentrated protein hydrolysates were low molecular weight peptides (90.2–90.7% dry matter) with all essential amino acids in a well-balanced state. Fish oil contained a large amount of valuable poly- and monounsaturated fatty amino acids (extraction ratio of 86.8–88.1%) and omega-3 fatty acids (10.82 and 22.32%, depending on the type of hydrolysis). Sludge was a protein-mineral product containing water-insoluble proteins (53.6–26.9% dry matter), fat (15.6–24.0%) with valuable poly- and monounsaturated fatty amino acids (15.0–24.5%), a high amount of omega-3 fatty acids (8.46 and 20.73%, depending on the type of hydrolysis), as well as minerals, mostly calcium and phosphorus (29.4–48.1%).

Thermal hydrolysis, which is simpler and faster, allowed 39.6% of protein to be extracted from raw materials in the form of protein hydrolysate with a protein content of 92%. The protein fraction with a molecular weight of more than 20 kDa accounted for 49.1%, and less than 10 kDa, 36.7%. The fat extraction rate of lipids into fish oil was 86.8%. In the protein-mineral by-product, protein content was 53.6%, mineral content was 29.4% and fat content was 15.9%, with protein recovery rate of 60.6% and mineral recovery rate of 91.1%. Thermal hydrolysis resulted in a more balanced amino acid and fatty acid composition compared to enzymatic-thermal hydrolysis.

Enzymatic-thermal hydrolysis is more labor-intensive but made it possible to extract 83% of protein from raw materials in the form of protein hydrolysate with a protein content of 92.6%, which in terms of the amino acid and fatty acid balance exceeded similar products after thermal-hydrolysis. The fraction of proteins with a molecular weight of more than 20 kDa accounted 6.7% and with that of less than 10 kDa, 87.6%. The fat extraction rate was higher than during thermal hydrolysis, with 88.1% of lipids. The resulting protein-mineral by-product had a reduced biological value in terms of amino-acid and fatty acid composition compared to the by-product obtained by thermal hydrolysis.

The high nutritional value of all three products after thermal and combined hydrolysis allows them to be used as food and feed additives – sources of valuable amino acids, fatty acids and minerals. These additives should be used for nutritional purposes separately or as components of food products (bakery and confectionery products). For feed purposes, hydrolysis products are recommended to be introduced into the composition of fish, farm animals and poultry feed.

Additionally, there is a possibility of using unrefined small underutilized fishes with lower fat content through hydrolysis processing in novel food technologies to obtain protein products with high biological value [19]. Further research should be done in this field as well. Furthermore, combination of fish hydrolysates with plant origin protein and anti-oxidant component may improve functional and economic characteristics of new products with high biological value [20, 21].

CONTRIBUTION

The authors were equally involved in the writing of the manuscript and are equally responsible for any potential plagiarism.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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