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SUPPLEMENTARY MATERIALS: Antioxidant and ACE I inhibitory peptides, DNA content and physico-chemical composition, with special focus on trace elements and pollutants, of spring NVG Atlantic herring (*Clupea harengus*) milt and hydrolysates.

Table S1. Total amino acid profile of the NVG herring milt and hydrolysates. Results of n=3 separate UPLC analysis. ds= dry sample

Amino Acid residue	HMC [g/ 100 g ds]	H1 [g/ 100 g ds]	H2 [g/ 100 g ds]	H3 [g/ 100 g ds]	FAO/WHO/UNU (2007)
Non - Essential					
Ala[a]	4.96±0.01	2.79±0.10	2.78±0.06	4.10±0.04	
Arg	27.13±0.07	10.53±0.17	20.27±0.78	23.96±0.52	
Asn	1.23±0.01	1.92±0.02	1.04±0.07	3.22±0.07	
Asp	5.52±0.08	3.07±0.11	2.61±0.07	3.91±0.06	
Cys*	0.23±0.00	0.17±0.00	0.07±0.01	0.07±0.02	
Glu	8.17±0.23	4.96±0.13	3.93±0.10	4.85±0.12	
Gly[a]	6.71±0.10	4.47±0.08	4.19±0.16	6.91±0.02	
Orn	0.00	0.00	0.00	0.25±0.00	
HyPro	0.33±0.06	0.00	0.00	0.00	
Pro[a]	5.65±0.07	2.90±0.06	3.24±0.05	3.63±0.07	
Ser	4.79±0.06	2.85±0.05	3.29±0.04	4.58±0.06	
Tau	2.61±0.00	2.48±0.03	1.88±0.09	4.02±0.03	
Tyr	2.94±0.06	1.99±0.24	2.58±0.06	4.34±0.01	
NVal	0.00	0.00	0.00	0.00	
Essential					
His	2.16±0.10	1.54±0.02	0.74±0.04	2.02±0.04	15
Ile[a]	3.33±0.01	2.51±0.04	2.15±0.04	3.33±0.08	30
Leu[a]	6.10±0.02	4.62±0.11	3.66±0.05	6.08±0.07	59
Lys	4.10±0.08	1.89±0.02	1.84±0.07	1.67±0.01	45
Phe[a]	4.27±0.17	4.88±0.05	3.16±0.06	6,39±0.05	22**
Met[a]	3.08±0.05	3.79±0.07	2.03±0.08	3.61±0.00	38***
Thr	4.01±0.07	2.93±0.02	2.55±0.02	3.77±0.31	23
Trp#	nd	nd	Nd	Nd	6
Val[a]	4.74±0.02	3.20±0.01	3.02±0.07	4.73±0.10	39
ΣTAA	102.04±0.06	63.51±0.07	63.03±0.10	95,45±0.08	
EAA (%)	31.78	25.36	19.15	31.61	
NEAA (%)	70.27	38.15	45.88	63.85	
Ratio EAA/NEAA	0.45	0,66	0,42	0,50	
Ratio LYS/ARG	0.15	0,18	0,09	0,07	
Total Hydrophobic Amino Acids	38.80±0.06	29.20±0.06	24.20±0.08	38.8±0.07	

*Cys is only partially recovered with the analytical method used

**Reference value for Phe+Tyr
***Reference value for Met+Cys (16 + 6)
EAA = Essential amino acids
NEAA = non-essential amino acids
a = Hydrophobic amino acids
#Tryptophane is destroyed by the acid hydrolysis used by us for the UPLC analysis

Table S2. Amino acid score (AAS) and essential amino acid index (EAAI) of NVG herring (*Clupea harengus*) milt and hydrolysates. ds=dry sample.

Amino Acid residue	Amino Acids scoring pattern	HMC [g/ 100 g ds]	H1 [g/ 100 g ds]	H2 [g/ 100 g ds]	H3 [g/ 100 g ds]
His	15	2.16±0.10	1.54±0.02	0.74±0.04	2.02±0.04
Ile	30	3.33±0.01	2.51±0.04	2.15±0.04	3.33±0.08
Leu	59	6.10±0.02	4.62±0.11	3.66±0.05	6.08±0.07
Lys	45	4.10±0.08	1.89±0.02	1.84±0.07	1.67±0.01
Met + Cys	22	3.31±0.05	3.96±0.07	2.10±0.09	3.68±0.02
Phe + Tyr	38	7.21±0.23	6.87±0.29	5.74±0.12	10.73±0.06
Thr	23	4.01±0.07	2.93±0.02	2.55±0.02	3.77±0.31
Trp	6	0.00	0.00	0.00	0.00
Val	39	4.74±0.02	3.20±0.01	3.02±0.07	4.73±0.10
LAA*	---	His	His	His	Lys
AAS (%)	---	14.4	14.4	14.4	3.71
EAAI (%)	---	108.71	91.91	83.91	112.12

*LAA = limiting amino acid

Table S3. Free amino acid profile of the NVG herring milt and hydrolysates by UPLC analysis (n=3). ds= dry sample

Amino Acid residue	HMC [g/ 100 g ds]	H1 [g/ 100 g ds]	H2 [g/ 100 g ds]	H3 [g/ 100 g ds]
Non - Essential				
Ala	0.065±0.001	0.170±0.003	0.287±0.004	0.405±0.014
Arg	0.331±0.004	0.643±0.001	0.804±0.010	0.865±0.006
Asn	0.020±0.000	0.052±0.001	0.161±0.002	0.235±0.002
Asp	0.027±0.001	0.169±0.000	0.265±0.004	0.341±0.017
Cys*	n.d.	0.013±0.001	0.034±0.002	0.040±0.001
Glu	0.051±0.001	0.195±0.009	0.262±0.001	0.396±0.015
Gly	0.268±0.004	0.242±0.003	0.331±0.001	0.357±0.011
Orn	0.003±0.001	0.017±0.001	0.006±0.001	0.006±0.001
HyPro	n.d.	0.004±0.000	0.009±0.000	n.d.
Pro	0.028±0.001	0.059±0.000	0.044±0.001	0.043±0.002
Ser	0.050±0.000	0.246±0.004	0.353±0.004	0.456±0.002
Tau	0.653±0.006	0.376±0.002	0.347±0.001	0.533±0.011
Tyr	0.030±0.001	0.185±0.003	0.344±0.005	0.456±0.016
NVal	n.d.	n.d.	n.d.	n.d.
Essential				
His	0.030±0.001	0.053±0.001	0.056±0.000	0.160±0.001
Ile	0.018±0.001	0.080±0.001	0.273±0.001	0.340±0.002

Leu	0.030±0.001	0.313±0.003	0.542±0.003	0.682±0.001
Lys	0.021±0.000	0.103±0.000	0.256±0.000	0.407±0.020
HyLys1	n.d.	0.003±0.000	0.003±0.000	0.007±0.000
HyLis2	n.d.	0.037±0.001	n.d.	n.d.
Phe	0.022±0.001	0.270±0.003	0.409±0.006	0.551±0.004
Met	0.041±0.002	0.047±0.034	0.217±0.001	0.286±0.008
Thr	0.033±0.001	0.140±0.002	0.281±0.003	0.387±0.000
Trp#	n.d.	0.076±0.005	0.078±0.003	0.156±0.004
Val	0.033±0.001	0.102±0.000	0.375±0.002	0.479±0.003
ΣTFAA	1.752±0.001	3.594±0.004	5.737±0.002	7.579±0.006
FEAA	0.496±0.001	1.223±0.005	2.490±0.002	3.446±0.004
FNEAA	1.256±0.002	2.371±0.002	3.247±0.003	4.133±0.008
Ratio FEAA/FNEAA	0.40	0.52	0.77	0.83

*Cys is only partially recovered with the analytical method used
n.d. = not detected

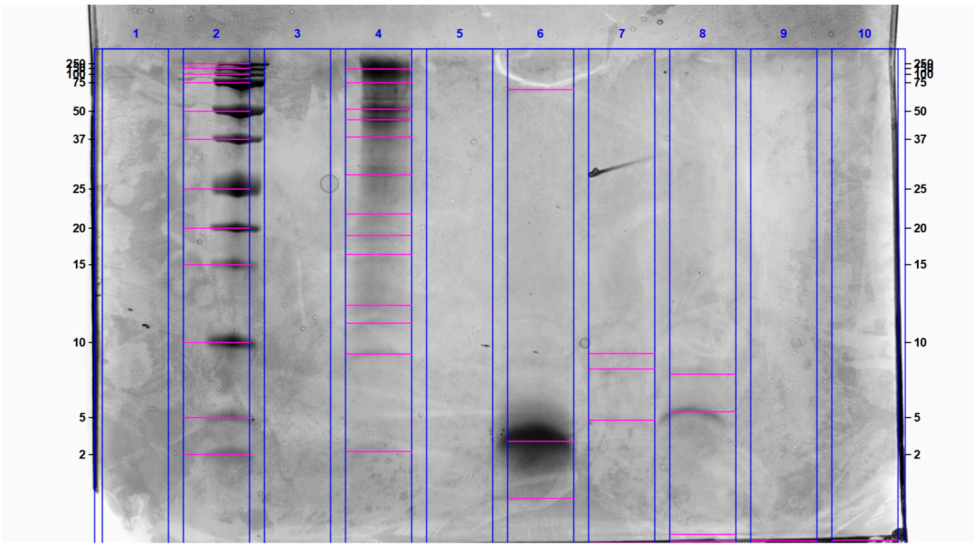
Figure S1 (hybrid). Peptide profile of herring milt and hydrolysates by electrophoretic analysis, by use of the BioRad Mini Protean system. As molecular weight marker Precision Plus Protein© Dual Xtra Standard of BioRad was applied (Catalogue #161-0377). Staining was done with Coomassie Blue R.

A) Distribution of the molecular weights per sample after native electrophoresis (PAGE) in a 10% MP TGX gel, as calculated by the Image Lab 6.1 software (BioRad).

B) 16.5% SDS-PAGE Tris-Tricine electropherogram of the herring milt and hydrolysates, stained with Coomassie Blue R: lane 2 – MW Standards [10 µl]; lane 4 – sample HMC [20 µl], lane 6 – sample H1 [20 µl], lane 7 - sample H2 [20 µl]; lane 8 - sample H3 [20 µl].

HMC Band Molecular Weight [kDa]	Band % (by intensity)	H1 Band Molecular Weight [kDa]	Band % (by intensity	H2 Band Molecular Weight [kDa]	Band % (by intensity	H3 Band Molecular Weight [kDa]	Band % (by intensity
250	3.4	193.6	2.0	193.6	2.9	250.0	3.8
205	4.5	≤15.2	98.0	15.3	76.7	195.7	2.3
173,3	2.8			11.2	10.0	15.2	80.5
13,9	34.1			<10	10.4	<10.4	13.3
<10	55.1						

A)



B)