EMFAF PROGRAMME 2021-2027

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Small Laboratory Trials and Semi-Industrial Trial to Assess the Suitability of Production Technology for High-Protein Fish Protein Based on Bream (Abramis brama)

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Content

Background

The fisheries sector in the Lake Peipsi region is facing a growing challenge: a significant share of the available bream (Abramis brama) catch quota remains unused because current market demand and prices do not make harvesting economically attractive. As a result, valuable fish resources are underutilized, limiting income opportunities for fishers, traders, and processors.

This project addresses this challenge by exploring the feasibility of producing high-protein fish protein hydrolysate powder from bream through enzymatic processing. The development of new value-added products can create alternative markets for low-value or underutilized fish species, increasing their commercial value and supporting more efficient use of available fish stocks.

The project will generate practical knowledge on suitable processing technologies, product yield, nutritional composition, safety parameters, and market potential. These results will provide the fisheries and fish-processing sector with evidence-based information needed to assess the commercial viability of bream-derived protein ingredients for use in animal feed, food products, and pharmaceutical applications.

By creating new valorisation pathways for bream, the project has the potential to strengthen the economic sustainability of the fisheries sector, improve resource efficiency, and contribute to the development of innovative bio-based products from local aquatic resources.

Technology

Enzymatic hydrolysing can be applied to upgrade the several fractions present in the bream raw material.

These fractions can be obtained by treating the raw material with proteolytic enzymes, which cut up large protein chains in smaller di/tri-peptides and free amino-acids.
In this way part of the proteins will be dissolved as hydrolysate (SPH) whilst others will remain non dissolved as solids (PHP).
Lipid material will be separated in the same process (OIL).
The bones/scales fraction (BS) will be removed and washed on a vibrating sieve.

By conducting initial laboratory trials with 3 commercial enzymes it will be determined which enzyme is most suitable for conducting a semi-industrial trial.

Enzymes to be examined:
1. Alcalase 2.4 (Novonesis, DK)
2. Endocut 09-L (Tailorzymes,DK)
3. Corolase 8000 (AB Enzymes, D)

In the semi-industrial trial enzymatic hydrolysis will be applied with the preferred enzyme on whole bream, with appropriate pretreatment and subsequent separation into proteins, oil and solid fractions.

Goal:

- Produce a high yield of fish protein hydrolysate with low fat and ash content, suitable for human consumption (by spray-drying).
- Produce high-quality oil with minimal affection of the oil's oxidation level
- Sieved and washed bone/scales meal and non-soluble protein meal (by mill drying).
- Price-indication on the produced SPH

Trial set-up.

The whole bream for the trials, was supplied in frozen form, in blocks of about 15 kg. each.

Before processing, the raw material was thawed overnight and minced to 6 mm. in a meat grinder.
By doing so, the surface of the raw material is increased which is improving the effectiveness and yield of the enzymatic process.

Defrosted bream in grinder

Grinded bream

Lab trials:

For the lab trials, 1 kg. of minced material was mixed with 1 part of tap water and heated in a water bath to 55˚Celsius. At this temperature 0,1% of enzymes is added (calculated on the raw material) and mixed for 1 hour.
After 1 hour the temperature is increased to 92˚ C. in order to inactivate the enzymes.
Separation of the different fractions was done by an initial removal of the bones on a hand sieve (1 mm.) followed by a spinning in a Heraus lab centrifuge (10 min. at 4200 rpm.).

The separated fractions after centrifugation are weighted and from the SPH the dry matter is determined using a moisture balance.

Semi industrial trials:

In order to minimize oil-oxidation, anti-oxidant is added right during mincing at 6 mm.
The raw material is mixed with water inside the reactor and heated to 55° Celsius.
The amount of added water is decided upon the viscosity of the total mass.
This should be low enough to perform a low temperature oil extraction, which is the first separation step.

Note: The cold oil extraction was not performed in the lab trials.

After the cold oil-extraction, the oil is further refined in a lab centrifuge by heating to 92˚and centrifuging for 10 minutes at 4200 rpm.

The stickwater and solids are put back into the reactor and additional water is being dosed.
At 55° C., the enzymes are being added. Dosage of the enzymes is calculated on the raw material.
After 2 hours of hydrolysing, the content of the reactor is inactivated at 92° Celsius for 15 minutes.

The reason for doing so is 3-fold:

1. *Inactivation of the enzymes*. The enzyme activity is stopped when heated to 92° C. which minimizes the possible formation of bitter off-taste notes in the hydrolysate.
2. *Pasteurizing of the reactor content*. At 92° C. most micro-organisms are being killed (except spores).
3. *Liquifying the oil*. The higher the temperature, the easier oil can be separated.

After the 92° C. heating step, the content of the reactor is emptied over a 1 mm. vibrating sieve.
This separates the bones/scales and (most) attached solids from the liquid.

Hydrolysing reactor

Vibrating sieve

Next separation was done in an 3-phase decanter, in which additional oil (if any) and solids are being separated from the liquid coming from the sieve.

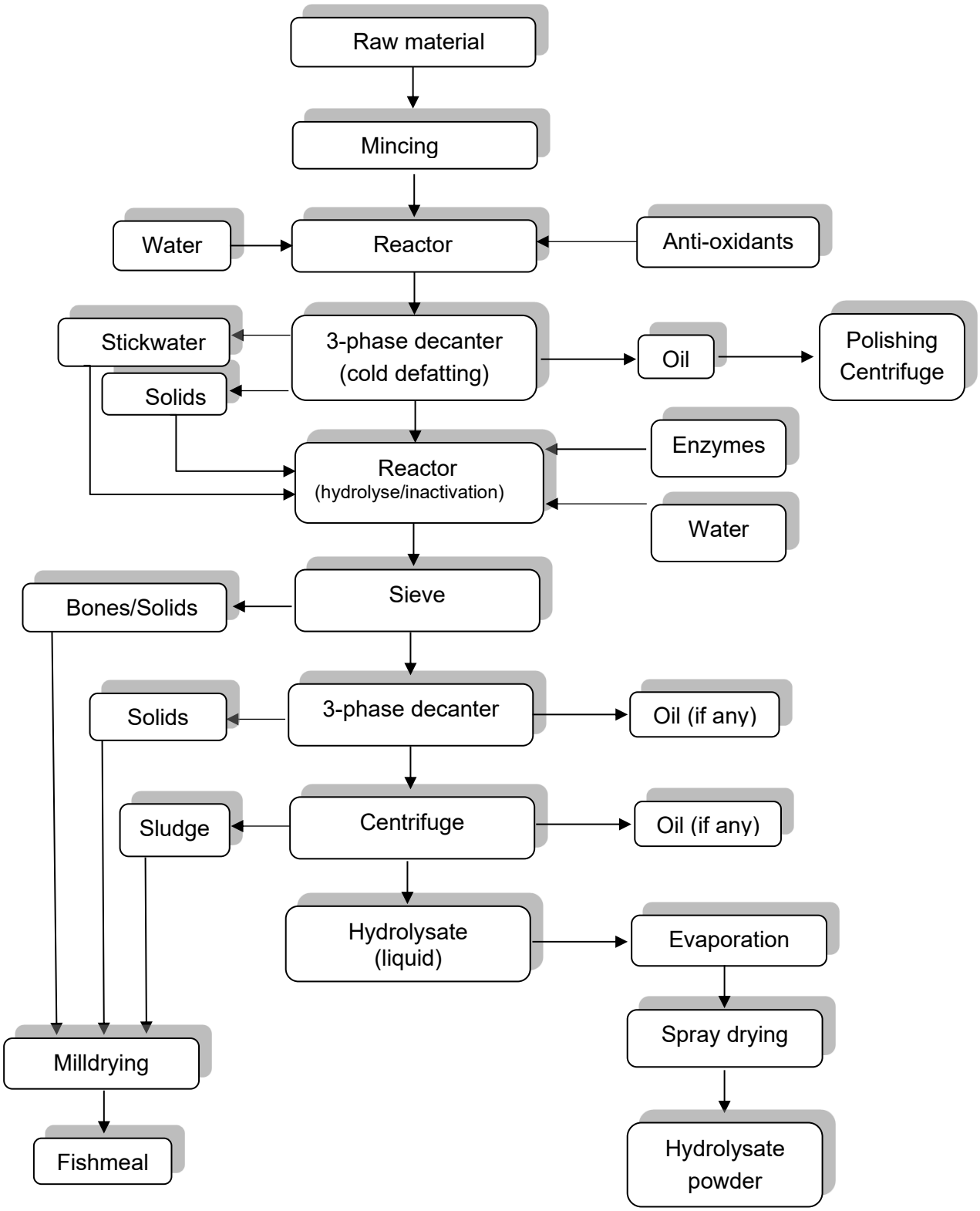
In a last process step, the hydrolysate was further refined in a centrifuge, in which additional solids ("sludge") and lipids are being minimized in the hydrolysate. Prior to this clarification step, the SPH liquid from the decanter was reheated to 92° C. again in a buffer tank.
This optimizes the separation in the centrifuge step.

3-phase decanter (GEA, CD220)

Centrifuge (GEA, ASE40)

In order to stabilize the hydrolysate, it was evaporated and finally spray dried.

Process flow hydrolysing bream



Results.

Lab trials:

There were some differences in the yield of the various enzymes.

Hydrolyzing whole bream; lab scale

Test 1: Corolase 8000		
Raw material in	gram	%
Grinded bream	1000	50,0
Water	1000	50,0
Corolase 8000	1	0,10
TOTAL	2001	100

Material out	gram	%	d.s. %	Yield%
BS	770	38%		
SPH	1155	58%	9,0	10,4
Oil	76	4%		
PHP	0	0%		
TOTAL	2001	100%		

Test 2: Alcalase 2.4L		
Raw material in	gram	%
Grinded bream	1000	50,0
Water	1000	50,0
Alcalase 2.4 L	1	0,10
TOTAL	2001	100

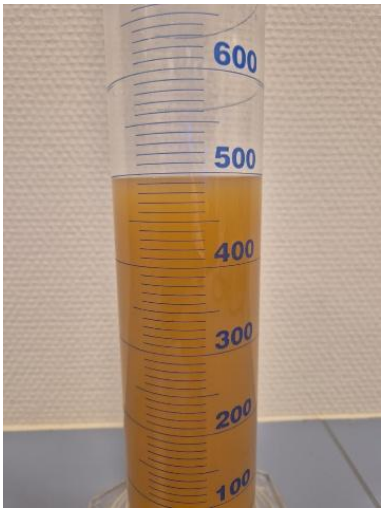
Material out	gram	%	d.s. %	Yield%
BS	760	38%		
SPH	1160	58%	8,90	10,3
Oil	55	3%		
PHP	25	1%		
TOTAL	2000	100%		

Test 3: Endocut 09-L		
Raw material in	gram	%
Grinded bream	1000	50,0
Water	1000	50,0
Endocut 09-L	1	0,10
TOTAL	2001	100

Material out	gram	%	d.s. %	Yield%
BS	724	36%		
SPH	1225	61%	9,20	11,3
Oil	51	3%		
PHP	0	0%		
TOTAL	2000	100%		

The Endocut 09-L shows the highest yield in SPH, whilst the Corolase 8000 seems to be more effective on oil separation.

It is observed however that all 3 enzymes show no clear separation of hydrolysate and that quite some solids still remain undissolved and attached to the bones.



Unclear hydrolysate

Bones/scales from sieve (not washed)

Therefore it is decided to optimize the hydrolyzing process by adjusting some parameters:

1. Increasing of the enzyme dosage from 0,1% to 0,2%
2. Increasing the water dosage to 1 : 1,25 instead of 1 : 1
3. Doubling the hydrolyzing time to 2 hours

To minimize the risk of unwanted bitter hydrophobic peptides, a small amount of Exocut TRL is added.

In order to verify these adaptations, another lab trial was performed.

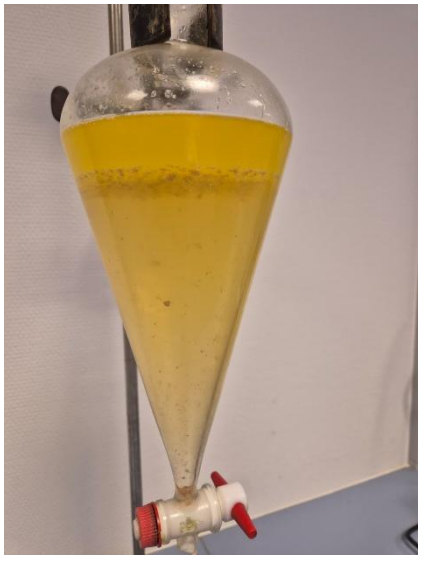
The result of this lab trial is stated below:

Test 4
Bream
Endocut 05/09-L
Dosage: 0,20%

SIEVE/CENTRIFUGE									
Input:	%	gr.	**Output:**	%	gr.	d.s.%	d.s. gr	Yield%
Bream	44,4%	1000,0	Bones (sieve)	5,9%	132,0	50%	66	6,6%
Water	55,6%	1250,0	PHP (centrifuge)	7,1%	160,0	25%	40	4,0%
			Oil (centrifuge)	2,9%	65,0	100%	65	6,5%
			Hydrolysate (centrifuge)	84,1%	1893,0	4,9%	92,8	9,3%
Total	100%	2250,0	Total	100%	2250,0			26,4%

It is obvious that the changes in the various parameters improves the effectiveness of the hydrolyzing process.

The bones/scales are more clean and the hydrolysate and oil came out brighter as well.
Non-dissolved proteins (PHP) were hardly noticeable.



Hydrolysate/oil lab test 4

Semi industrial scale trial:

As a first step, the minced bream was added into the reactor and heated to 55˚ C. after which sufficient water (also 55˚ C.) was added to deem a cold defatting possible in the decanter.
During mincing , a small amount of anti-oxidant was added to the minced bream.
Dosage was 0,1% of RG20 (Kemin), calculated on the raw material.

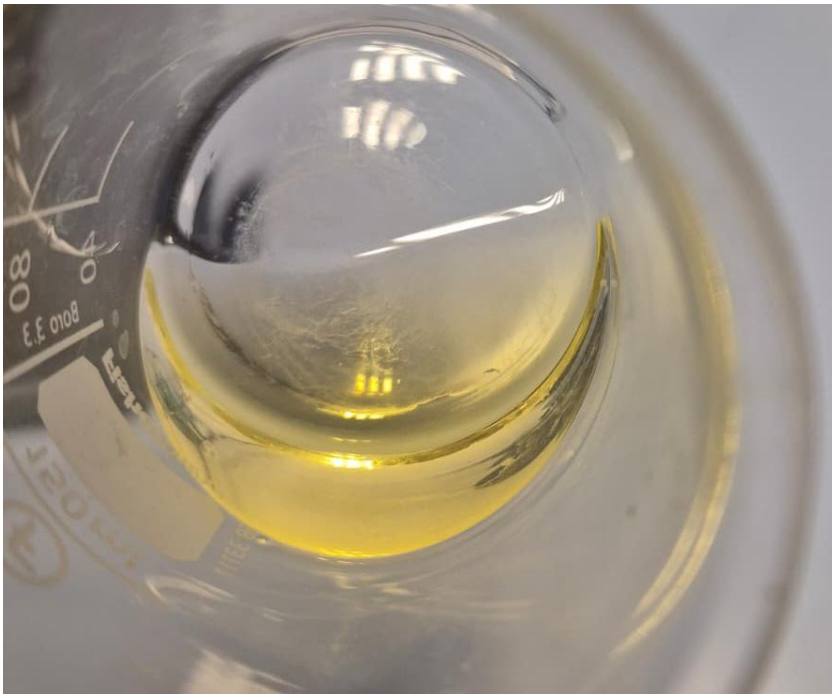
Hydrolyzing whole bream; semi industrial scale. Cold defatting step.

Raw material in	kg.	%
Grinded bream	400	66,6
Water	200	33,3
Anti-oxidant RG20	0,40	0,10
TOTAL	600	100

Material out	kg.	%	d.s. %	Yield%
Bones/solids	248	41%		
Stickwater	320	53%		
Oil (cold separated)	32	5%	97,00	7,8
TOTAL	600	100%		

A sample of the cold separated oil is heated to 92˚ C. and then separated again (“polished”) in a small centrifuge.

As a result the oil was purified and all present water/solid particles were removed.



Cold separated bream oil (after polishing).

In a second step, the separated solids and stick water from the decanter are added back into the reactor.

Note: Because the reactor ideally is filled with around 600 kg. total, not all stickwater and solid are added back into the reactor.

The 400 kg. re-added bones & stickwater originates from 268 kg. fresh bream.

The amount of water to be added is calculated bearing in mind that the initial raw material was already mixed with 50% water.

Based on the fact that a 1 : 1,25 has to be reached, additional water is added to the reactor and heated to 55˚ C. At this temperature the enzymes are added.
After 2 hours hydrolyzing the inactivation was done at to 92˚ C. after which the content of the reactor was emptied using a vibrating screen (1 mm.) to remove the bones.

The liquid was then pumped through the decanter and centrifuge (after intermediate re-heating).

Hydrolyzing whole bream; semi industrial scale.				**Decanter step**				
Raw material in	kg.	%		Material out	kg.	%	d.s. %	Yield. %
Bones/solids	176	29,1		BS (sieve)	35	6%	48,80	6,4
Stickwater	224	37,1		SPH (separator)	500	83%	4,50	8,4
Water	203	33,6		Oil (decanter)	0	0%		
Endocut 09-L/Exocut TRL	1,20	0,20		PHP (decanter)	43	7%	24,50	3,9
				PHP (separator)	26			
TOTAL	604	100		TOTAL	604	96%		18,7

The soluble hydrolysate is evaporated to a concentrate with 32,6% dry matter.

This concentrate is then spray dried into a powder with a dry matter of 95,1%

Further processing of washed bones/scales and PHP fraction:

In order to stabilize the non-soluble protein and the sieved bones/scales, a ULTRAROTOR system has been applied (Jäckering Germany) which dries and mills the incoming product in 1 step.

The basic principle

Inside the machine, a high-performance rotor rotates at high speed. At the same time, a downstream fan ensures a high through-put of air. This, in connection with the design of the inner milling chambers, creates extreme air turbulence.

Pulverizing

During the milling process, the turbulent air directs the particles in such a way that they collide with each other again and again. This causes them to break at their natural breaking points.

The coarseness can be adjusted, even down to the micron range. After the milling process, the milled product can be separated via downstream sieves or sifters if required.

Ultrarotor system Jäckering

Results of dry milling

A. Washed bones/scales from sieve.

Dry matter% of input: 36,6%

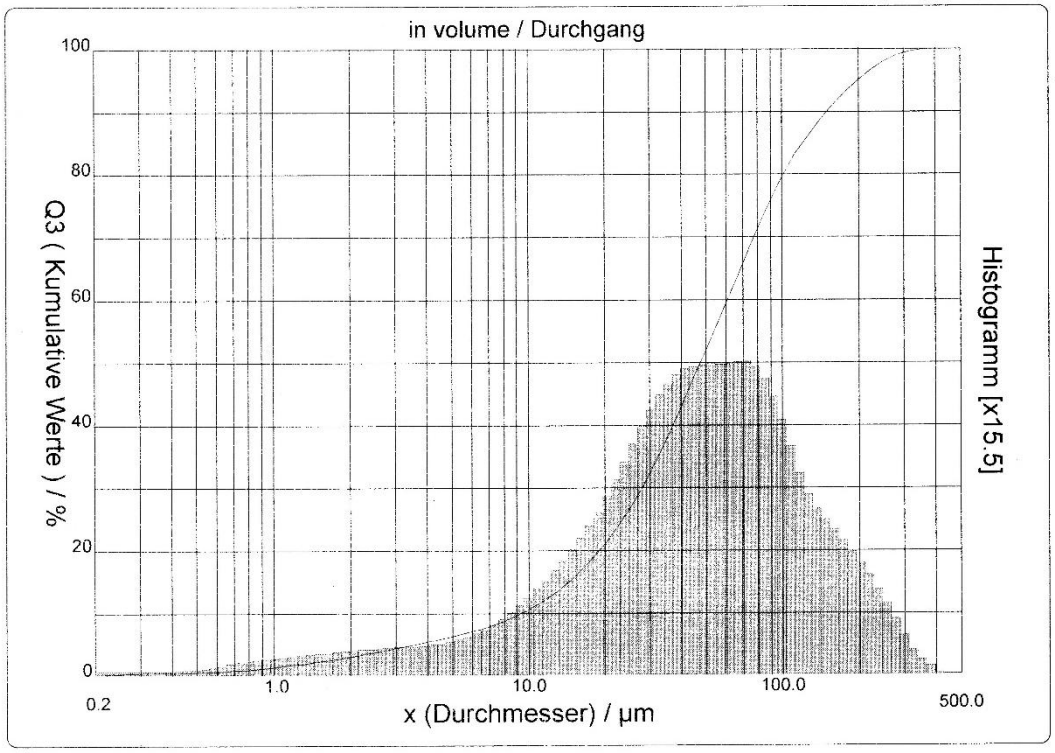
Dry matter% of output: 0,1%

Particle size 50% smaller than 47,55 micron

Particle size 90% smaller than 149,92 micron

Particle size 99% smaller than 284,68 micron

The CILAS curve below shows the particle size distribution.



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B. Solids from decanter (PHP).

Dry matter% of input: 76,5%

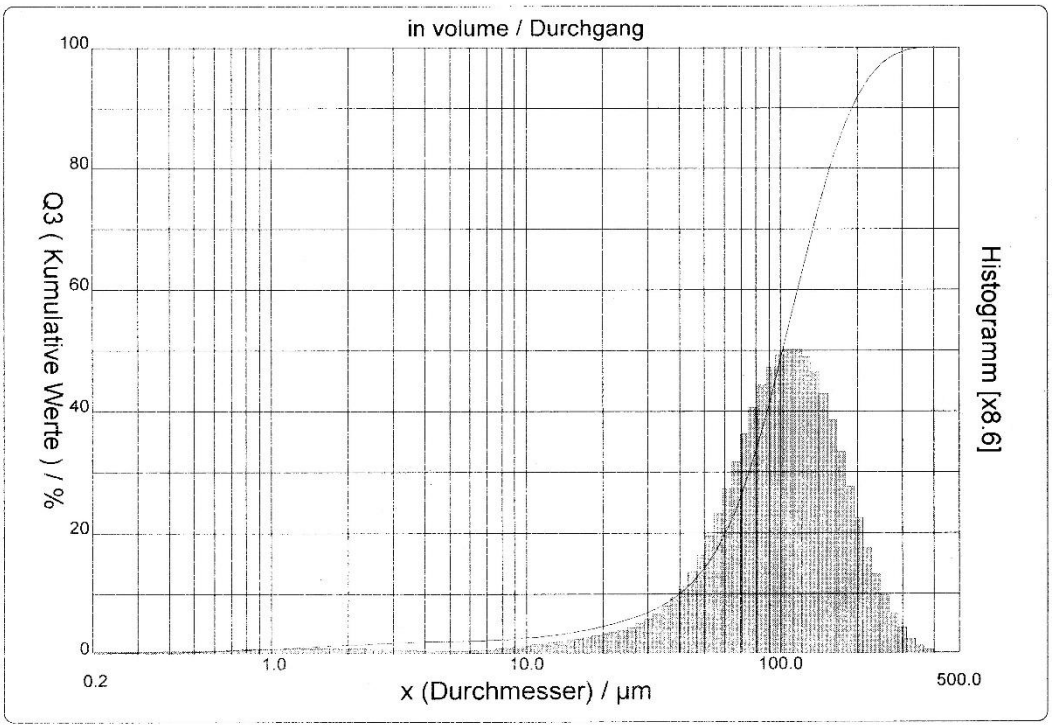
Dry matter% of output: 2,6%

Particle size 50% smaller than 101,81 micron

Particle size 90% smaller than 190,69 micron

Particle size 99% smaller than 288,26 micron

The CILAS curve below shows the particle size distribution.



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Analysis Report – Bream raw material

Sample Bream raw material
Sample number M26009780001
Receipt / sampling date 22-4-2026

Parameter	Result 1	Result 2	Mean	Unit	Method	Accred.
Moisture (103°C, 4 hrs, sand)	789	785	787	g/kg	10032	Q
Dry matter (103°C, 4 hrs, sand)	211	215	213	g/kg	10032	Q
Crude Protein (Kjeldahl, N=6.25)	119	117	118	g/kg	10005	Q
Crude Fat (by acid hydrolysis)	70	71	70,5	g/kg	10497	
Crude Ash (550°C)	24	22	23	g/kg	10028	Q
Minerals & salt						
Sodium (Na)	0,92			g/kg	10040	Q
Salt as NaCl (based on sodium)	2,35			g/kg	10040	
Freshness						
TVB-N (Total Volatile Basic Nitrogen)	140			mg/kg	10298	
Heavy metals						
Arsenic (As)	<0,050	<0,050	<0,050	mg/kg	10222	Q
Cadmium (Cd)	<0,010	<0,010	<0,010	mg/kg	10222	Q
Mercury (Hg)	0,015	0,016	0,0155	mg/kg	10222	Q
Lead (Pb)	<0,050	<0,050	<0,050	mg/kg	10222	Q

Analysis Report – Bream raw material

Sample Bream raw material
Sample number M26009780001
Receipt / sampling date 22-4-2026

Compound	Result	Unit	Method
PFAS (per- and polyfluoroalkyl substances)			
Perfluorohexanesulfonate (PFHxS)	<0,30	µg/kg	10577
Perfluorooctanesulfonate (PFOS)	0,60	µg/kg	10577
Perfluorooctanoic acid (PFOA)	<0,30	µg/kg	10577
Perfluorononanoic acid (PFNA)	0,31	µg/kg	10577
Sum PFOS/PFOA/PFNA/PFHxS (excl. LOQ)	<1,2	µg/kg	10577

Note: method code 10577. Values with '<' are below the reporting limit (LOQ). Sum is calculated excluding LOQ (lower-bound).

Analysis Report – Bream raw material

Sample	Bream raw material
Sample number	M26009780001
Receipt / sampling date	22-4-2026

Compound	Result	Unit	Method
TEQ summary (WHO 2005)			
WHO-TEQ (PCDD/F + DL-PCBs) incl. LOQ	3,282	ng TEQ/kg	10463
Dioxin TEQ (WHO 2005) incl. LOQ	1,680	ng TEQ/kg	10463
DL-PCB TEQ (WHO 2005) incl. LOQ	1,601	ng TEQ/kg	10463
Dioxins (PCDD)			
2,3,7,8-TCDD	0,17	ng/kg	10463
1,2,3,7,8-PeCDD	0,32	ng/kg	10463
1,2,3,4,7,8-HxCDD	0,19	ng/kg	10463
1,2,3,6,7,8-HxCDD	0,31	ng/kg	10463
1,2,3,7,8,9-HxCDD	<0,08	ng/kg	10463
1,2,3,4,6,7,8-HpCDD	<0,10	ng/kg	10463
OCDD	<0,20	ng/kg	10463
Furans (PCDF)			
2,3,7,8-TCDF	6,25	ng/kg	10463
1,2,3,7,8-PeCDF	0,78	ng/kg	10463
2,3,4,7,8-PeCDF	1,33	ng/kg	10463
1,2,3,4,7,8-HxCDF	0,31	ng/kg	10463
1,2,3,6,7,8-HxCDF	0,26	ng/kg	10463
2,3,4,6,7,8-HxCDF	0,19	ng/kg	10463
1,2,3,7,8,9-HxCDF	<0,08	ng/kg	10463
1,2,3,4,6,7,8-HpCDF	0,13	ng/kg	10463
1,2,3,4,7,8,9-HpCDF	<0,10	ng/kg	10463
OCDF	<0,20	ng/kg	10463
Non-ortho DL-PCBs			
PCB-77	67,17	ng/kg	10463
PCB-81	4,93	ng/kg	10463
PCB-126	13,27	ng/kg	10463
PCB-169	5,62	ng/kg	10463
Mono-ortho DL-PCBs			
PCB-105	718,4	ng/kg	10463
PCB-114	73,2	ng/kg	10463
PCB-118	1911,8	ng/kg	10463
PCB-123	39,3	ng/kg	10463
PCB-156	288,8	ng/kg	10463
PCB-157	51,1	ng/kg	10463
PCB-167	128,5	ng/kg	10463
PCB-189	29,5	ng/kg	10463

Note: all compounds determined under one method (code 10463, accredited).

Values with '<' are belowthe reporting limit (LOQ). TEQ values 'incl. LOQ' = upper-bound (LOQ included).

Analysis Report – Bream UT Estland – fraction comparison

Subject Bream UT Estland
Sampling date 22-4-2026

Parameter	Sieve solids	Decanter solids	Decanter liquid	Separator sludge	FPH liquid
	(2)	(3)	(4)	(5)	(6)
Composition (proximate)					
Moisture 103°C (4 hrs)	512	755	942	907	955
Dry matter 103°C (4 hrs)	488	245	58	93	45
Crude Protein (Kjeldahl, N=6,25)	190	211	46	77	39
Crude Fat (by acid hydrolysis)	6	23	8	12	3
Crude Ash (550°C)	284	11	4	5	4
Minerals & salt					
Sodium (Na)	1,96	0,90	0,76	0,81	0,75
Salt as NaCl (based on sodium)	4,98	2,28	1,94	2,06	1,91
Freshness					
TVB-N (Total Volatile Basic Nitrogen)	<50	120	110	110	–
Heavy metals					
Arsenic (As)	<0,050	<0,050	<0,050	<0,050	–
Cadmium (Cd)	<0,010	<0,010	<0,010	0,010	–
Mercury (Hg)	<0,010	0,041	<0,010	0,016	–
Lead (Pb)	0,117	<0,050	<0,050	<0,050	–

Units, methods & accreditation per parameter					
Parameter	**Unit**	**Method**	**Accr.**		
Moisture 103°C (4 hrs)	g/kg	10032	Q GMP		
Dry matter 103°C (4 hrs)	g/kg	10032	Q GMP		
Crude Protein (Kjeldahl, N=6,25)	g/kg	10005	Q GMP		
Crude Fat (by acid hydrolysis)	g/kg	10497 [1]	–		
Crude Ash (550°C)	g/kg	10028	Q GMP		
Sodium (Na)	g/kg	10040	Q GMP		
Salt as NaCl (based on sodium)	g/kg	10040	–		
TVB-N (Total Volatile Basic Nitrogen)	mg/kg	10298	–		
Arsenic (As)	mg/kg	10222	Q GMP+ QS		
Cadmium (Cd)	mg/kg	10222	Q GMP+ QS		
Mercury (Hg)	mg/kg	10222	Q GMP+ QS		
Lead (Pb)	mg/kg	10222	Q GMP+ QS		

Results reported exactly as issued by NutriControl (decimal comma). '<' = belowreporting limit (LOQ).

Accr.: Q = accredited (RvA, NEN-EN-ISO/IEC 17025, L053); GMP / GMP+ / QS = certification scope.

Analysis Report – Bream SPH oil

Subject	Bream UT Estland
Product	Bream FPH oil
Sampling date	22-4-2026

Parameter	Result	Unit
General composition		
Crude Protein (Kjeldahl, N=6,25)	<2	g/kg
Crude Fat	>999	g/kg
Crude Ash (550°C)	<1	g/kg
Oil quality & oxidation		
Anisidine number (AnV)	1,9	–
Peroxide value (PV)	2,4	meq O/kg
TOTOX value	6,7	–
Free fatty acids	10	g/kg
Acid value	1,9	mg KOH/g
Water content (Karl Fischer)	1,5	g/kg
Mean molecular weight of fatty acids	282	–
Fatty acid groups (g/kg)		
Elutable	863	g/kg
Saturated fatty acids	204	g/kg
Mono-unsaturated fatty acids	457	g/kg
Poly-unsaturated fatty acids	202	g/kg
Trans fatty acids	11	g/kg
Omega-3 fatty acids	118	g/kg
Omega-6 fatty acids	79	g/kg
Omega-9 fatty acids	228	g/kg

Methods & accreditation per parameter		
Parameter	**Method**	**Accr.**
Crude Protein (Kjeldahl, N=6,25)	10005	–
Crude Fat	10110	Q
Crude Ash (550°C)	10028	–
Anisidine number	10572	–
Peroxide value	10127	Q GMP
Mean molecular weight of fatty acids	10076	–
Free fatty acids	–	Q GMP
Acid value	–	Q GMP
Water content (Karl Fischer)	10269	Q GMP
Fatty acid profile	10091	Q GMP

Method descriptions (as stated in report)

10076 – Fats and oils per NEN-EN-ISO 660 (extraction: in-house method)

10091 – In-house method

10110 – In-house method

10127 – Fats and oils per NEN-EN-ISO 3960 (extraction: in-house method)

10269 – Equivalent to NEN-EN-ISO 8534 (lactose: in-house method)

Results reported exactly as issued by NutriControl (decimal comma). '<' / '>' = below/ above reporting limit.

Accr.: Q = accredited (RvA, NEN-EN-ISO/IEC 17025, L053);

GMP = GMP+ Feed Safety Assurance certification scope.

Bream SPH oil – fatty acid profile

Fatty acid	g/kg	Fatty acid	g/kg
C4:0 (butyric acid)	<1	C18:1n9 (oleic acid)	212
C5:0 (valeric acid)	<1	C18:1n others (inc. octadecenoic acid)	65
C6:0 (caproic acid)	<1	C18:2 trans (inc. linolelaidic acid)	5
C7:0 (enanthic acid)	<1	C18:2n6 (linoleic acid)	46
C8:0 (caprylic acid)	<1	C18:2 CLA-9c,11t (rumenic acid)	<1
C9:0 (pelargonic acid)	<1	C18:2 CLA-10t,12c (conjugated linoleic acid)	<1
C10:0 (capric acid)	<1	C18:3n3 (alfa-linolenic acid)	25
C10:1 (decenoic acid)	<1	C18:3n6 (gamma-linolenic acid)	3
C11:0 (undecylic acid)	<1	C18:4n3 (stearidonic acid)	5
C12:0 (lauric acid)	2	C19:0 (nonadecylic acid)	<1
C12:1 (lauroleic acid)	<1	C20:0 (arachidic acid)	2
C14:0 iso (isomyristic acid)	1	C20:1n9 (gondoic acid)	4
C14:0 (myristic acid)	22	C20:1n11 (gadoleic acid)	15
C14:1n5 (myristoleic acid)	2	C20:2n6 (dihomo-linoleic acid)	5
C14:1n9 (tetradecenoic acid)	7	C20:3n3 (dihomo-alfa-linolenic acid)	3
C15:0 iso (isopentanoic acid)	4	C20:3n6 (dihomo-gamma-linoleic acid)	4
C15:0 ante-iso (ante-isopentanoic acid)	3	C20:4n3 (eicosatetraenoic acid)	5
C15:0 (pentadecanoic acid)	4	C20:4n6 (arachidonic acid)	19
C15:1 (pentadecenoic acid)	4	C20:5n3 EPA (eicosapentaenoic acid)	47
C16:0 iso (isopalmitic acid)	<1	C21:0 (heneicosylic acid)	<1
C16:0 ante-iso (ante-isopalmitic acid)	<1	C22:0 (behenic acid)	<1
C16:0 (palmitic acid)	124	C22:1n9 (erucic acid)	<1
C16:1n7 (palmitoleic acid)	128	C22:1n11 (cetoleic acid)	<1
C16:1n9 (hexadecenoic acid)	4	C22:2n6 (docosadienoic acid)	<1
C16:3n3 (hexadecatrienoic acid)	<1	C22:3n3 (docosatrienoic acid)	<1
C16:4n3 (hexadecatetraenoic acid)	6	C22:4n6 (adrenic acid)	<1
C17:0 iso (isomargaric acid)	1	C22:5n3 DPA (docosapentaenoic acid)	12
C17:0 ante-iso (ante-isomargaric acid)	10	C22:5n6 (osbond acid)	2
C17:0 (margaric acid)	4	C22:6n3 DHA (docosahexaenoic acid)	16
C17:1 (heptadecenoic acid)	8	C23:0 (tricosylic acid)	<1
C18:0 iso (isostearic acid)	1	C24:0 (lignoceric acid)	<1
C18:0 (stearic acid)	25	C24:1n9 (nervonic acid)	<1
C18:1 trans (inc. elaidic acid)	6		

Results reported exactly as issued by NutriControl (decimal comma). '<1' = belowreporting limit. Profile method 10091, accredited (Q GMP).

Analysis Report – Bream SPH powder

Subject	Bream UT Estland
Product	Bream FPH powder
Sampling date	22-4-2026

Parameter	Result	Unit
Composition		
Moisture 103°C (4 hrs)	49	g/kg
Crude Protein (Kjeldahl, N=6,25)	794	g/kg
Crude Fat (by acid hydrolysis)	80	g/kg
Crude Ash (550°C)	76	g/kg
Minerals & salt		
Sodium (Na)	14,9	g/kg
Salt as NaCl (based on sodium)	37,9	g/kg
Freshness		
TVB-N (Total Volatile Basic Nitrogen)	1920	mg/kg
Heavy metals		
Arsenic (As)	0,162	mg/kg
Cadmium (Cd)	0,082	mg/kg
Mercury (Hg)	0,057	mg/kg
Lead (Pb)	<0,050	mg/kg
Biogenic amines		
Biogenic amines (upper bound)	258	mg/kg
Agmatine	<10	mg/kg
Cadaverine	59	mg/kg
Histamine	23	mg/kg
Phenylethylamine	<10	mg/kg
Putrescine	81	mg/kg
Spermidine	<35	mg/kg
Spermine	<30	mg/kg
Tyramine	<10	mg/kg

Methods & accreditation per parameter		
Parameter / group	**Method**	**Accr.**
Moisture 103°C	10032	Q GMP
Crude Protein (Kjeldahl)	10005	Q GMP
Crude Fat (acid hydrolysis)	10497	–
Crude Ash (550°C)	10028	Q GMP
Sodium / Salt	10040	Q GMP / –
TVB-N	10298	–
Heavy metals (As, Cd, Hg, Pb)	10222	Q GMP+ QS
Biogenic amines	10020	Q GMP [1]

[1] Biogenic amines accredited (Q GMP) except Spermine and the upper-bound sum.

Results reported exactly as issued by NutriControl (decimal comma). '<' = belowreporting limit (LOQ).

Accr.: Q = accredited (RvA, NEN-EN-ISO/IEC 17025, L053); GMP / GMP+ / QS = certification scope.

Bream SPH powder – amino acids & protein characterization		
Parameter	**Result**	**Unit**
Amino acid profile (method 10018, Q GMP)		
Alanine	54,5	g/kg
Arginine	46,2	g/kg
Aspartic acid	70,3	g/kg
Cysteine	5,4	g/kg
Glutamic acid	110,3	g/kg
Glycine	70,7	g/kg
Histidine	15,1	g/kg
Hydroxyproline	16,2	g/kg
iso-Leucine	28,9	g/kg
Leucine	52,5	g/kg
Lysine	64,1	g/kg
Methionine	19,7	g/kg
Phenylalanine	27,5	g/kg
Proline	40,8	g/kg
Serine	33,1	g/kg
Threonine	30,3	g/kg
Tyrosine	19,3	g/kg
Valine	32,6	g/kg
Protein characterization – GPC weight distribution (method 10536)		
Class 1	2,0	% a/a
Class 2	12,8	% a/a
Class 3	33,5	% a/a
Class 4	40,6	% a/a
Class 5	11,1	% a/a
Class 6	n.b.	% a/a
Number average molecular weight (Mn)	1928	Da
Weight average molecular weight (Mw)	5710	Da
Polydispersity	2,96	–

GPC class ranges (from report): .

Class 1 = 30 000–140 000 Da

Class 2 = 11 000–30 000 Da

Class 3 = 3 000–11 000 Da

Class 4 = 850–3 000 Da

Class 5 = 400–850 Da

Class 6 range not specified in report.

n.b. = not determined / belowdetection. Polydispersity = Mw/ Mn.

Results reported exactly as issued by NutriControl (decimal comma).

Bream SPH powder – dioxins & DL-PCBs		
Compound	**Result**	**Unit**
TEQ summary (WHO 2005)		
WHO-TEQ (PCDD/F + DL-PCBs) incl. LOQ	0,804	ng TEQ/kg
Dioxin TEQ (WHO 2005) incl. LOQ	0,438	ng TEQ/kg
DL-PCB TEQ (WHO 2005) incl. LOQ	0,366	ng TEQ/kg
Dioxins (PCDD)		
2,3,7,8-TCDD	0,05	ng/kg
1,2,3,7,8-PeCDD	0,08	ng/kg
1,2,3,4,7,8-HxCDD	<0,08	ng/kg
1,2,3,6,7,8-HxCDD	<0,08	ng/kg
1,2,3,7,8,9-HxCDD	<0,08	ng/kg
1,2,3,4,6,7,8-HpCDD	<0,10	ng/kg
OCDD	<0,20	ng/kg
Furans (PCDF)		
2,3,7,8-TCDF	1,51	ng/kg
1,2,3,7,8-PeCDF	0,21	ng/kg
2,3,4,7,8-PeCDF	0,31	ng/kg
1,2,3,4,7,8-HxCDF	0,10	ng/kg
1,2,3,6,7,8-HxCDF	<0,08	ng/kg
2,3,4,6,7,8-HxCDF	<0,08	ng/kg
1,2,3,7,8,9-HxCDF	<0,08	ng/kg
1,2,3,4,6,7,8-HpCDF	<0,10	ng/kg
1,2,3,4,7,8,9-HpCDF	<0,10	ng/kg
OCDF	<0,20	ng/kg
Non-ortho DL-PCBs		
PCB-77	15,35	ng/kg
PCB-81	1,20	ng/kg
PCB-126	3,00	ng/kg
PCB-169	1,28	ng/kg
Mono-ortho DL-PCBs		
PCB-105	179,0	ng/kg
PCB-114	15,7	ng/kg
PCB-118	465,0	ng/kg
PCB-123	15,2	ng/kg
PCB-156	72,8	ng/kg
PCB-157	12,7	ng/kg
PCB-167	70,9	ng/kg
PCB-189	7,6	ng/kg

Method 10463 (accredited, Q GMP+ QS). Values with '<' are belowthe reporting limit (LOQ).

TEQ values 'incl. LOQ' = upper-bound (LOQ included). Decimal comma as reported.

Bream SPH powder – PFAS		
Compound	**Result**	**Unit**
PFAS (per- and polyfluoroalkyl substances) – external		
Perfluorohexanesulfonate (PFHxS)	< 0,1	µg/kg
Perfluorooctanesulfonate (PFOS)	< 0,1	µg/kg
Perfluorooctanoic acid (PFOA)	< 0,1	µg/kg
Perfluorononanoic acid (PFNA)	1,9	µg/kg
Sum PFOS/PFOA/PFNA/PFHxS (excl. LOQ)	nb	µg/kg

Analysis subcontracted (external). '<' = belowreporting limit (LOQ); 'nb' = not calculable (sum excl. LOQ).

Results reported exactly as issued by NutriControl (decimal comma).

Conclusions & remarks

Process technology

The intended production technology – cold defatting, enzymatic hydrolysis, separation over sieve, decanter and separator, followed by evaporation and spray drying – has been shown to be feasible at semi-industrial scale on whole bream (*Abramis brama*).

The process steps can be designed in such a way that 4 separable fractions are produced:
A high-quality protein hydrolysate, an oil, a bone/solids fraction and a non soluble protein fraction.

Yield and quality of the hydrolysate

The optimisation (enzyme dosage 0.1 → 0.2%, water ratio 1 : 1.25 and hydrolysis time 2 hours) led to a cleaner process with a brighter hydrolysate and oil and hardly any noticeable non-dissolved protein.
The dry-matter yield of the hydrolysate appears to fall (from approximately 11% in the laboratory trials to 9.3% in Test 4), but this is not a deterioration: in the laboratory trials part of the protein remained non-dissolved and was still included in the dry-matter determination of the SPH. During scale-up this non-dissolved fraction (PHP) is separated off, leaving a considerably more soluble and pure hydrolysate.
The GPC analysis confirms this profile: the hydrolysate consists predominantly of small and medium-sized peptides (class 4: 40.6%, class 3: 33.5%, class 5: 11.1%), with a weight-average molecular weight of 5,710 Da and a number-average of 1,928 Da.

Note on the mass balance (semi-industrial): *Because of the maximum reactor volume, only part of the solids and stickwater could be re-dosed to the hydrolysis step after cold defatting (176 kg solids + 224 kg stickwater). The yields in the decanter step are therefore calculated on the quantity actually processed, corresponding to approximately 268 kg of fresh bream, and not on the original batch.*

Product composition

The SPH powder meets the objective of a high-protein product: 79.4% crude protein, 8.0% fat, 7.6% ash.
The salt content is relatively high (3.8% NaCl), which is relevant for application and labelling.
The oil is of good quality with a very low oxidation level (TOTOX 6.7) and a favourable fatty-acid profile with 118 g/kg omega-3 (EPA 47, DHA 16, DPA 12 g/kg).
The addition of anti-oxidant during mincing effectively limited oxidation.

Food safety

For the parameters tested, both raw material and powder comply with the EU maximum levels (Regulation 2023/915). Heavy metals, dioxins/DL-PCBs, PFAS and biogenic amines (histamine 23 mg/kg) lie well below the applicable limits in the powder; the WHO-TEQ falls from 3.28 ng/kg in the raw material to 0.80 ng/kg in the powder, because the lipophilic contaminants are separated off with the oil fraction.

Suitability for human consumption

On the basis of the parameters tested, the hydrolysate is in principle suitable for food-grade application.

Price indication

Based on market indications, the sales-price of this kind of food grade fish protein ranges from € 5 to € 12 per kg.