

Production of bioactive peptides from salmon processing side-streams



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Management

15-18 JUNE 2022



Optimal utilization of seafood side-streams through the design of new holistic process lines

The overall goal of the project is to evaluate processes for production of new ingredients and/or valuable products based on solids and liquid side-streams from the fisheries and aquaculture value chain which have been stored in an improved manner compared to state-of-the-art.

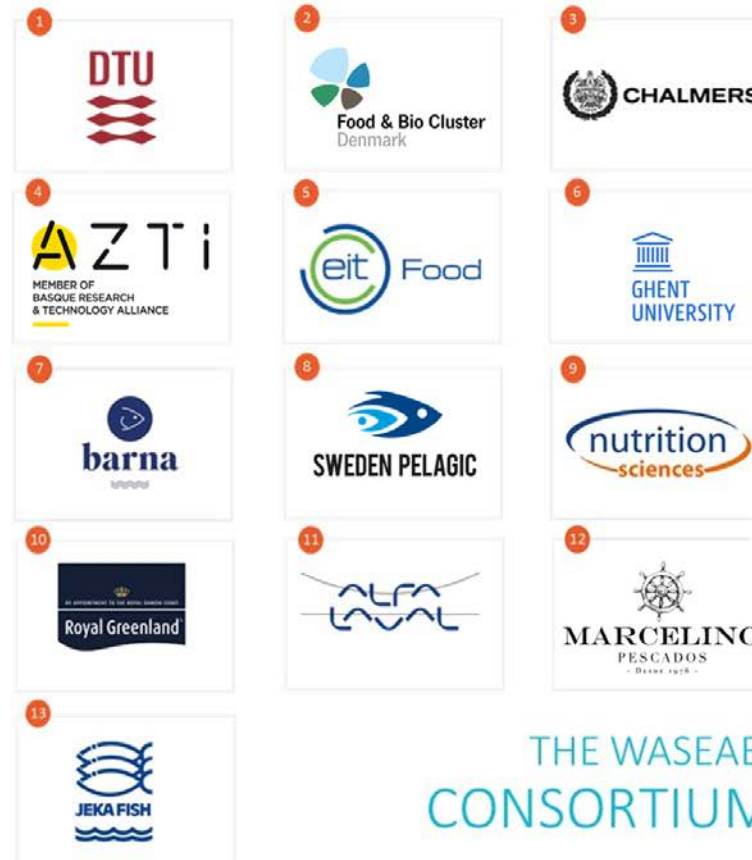


<https://www.waseabi.eu/>



@WaSeaBi

This project has received funding from the **Bio Based Industries Joint Undertaking (JU)** under the European Union's Horizon 2020 research and innovation programme under grant agreement **No 837726**. The JU receives support from the European Union's Horizon 2020 research and innovation programme and the Bio Based Industries Consortium



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Side-streams	Process	Targeted products
1-Mussel cooking water 2-Herring process water 3-Cod brine	Flocculation with centrifugation	Soluble proteins and lipids
4-Mussel cooking water	Concentration technologies	Savoury compounds
5-Cod brine 6-Solid cod side-streams 7-Liquid herring side-streams 8-Solid herring side-streams 9-Mussel cooking water	pH-shift technology	Protein isolates
10-Discards 11-Salmon solid side stream 12-Mussel shells 13-Cod solid side streams 14-Herring solid side streams	Enzymatic hydrolysis	Bioactive peptides
15-Cod solid side-streams	Enzymatic hydrolysis	Flavouring agents
16-Salmon solid side-streams		
17-Bones from Discards 18-Salmon bones	Enzymatic hydrolysis / alkaline treatment	Mineral ingredients
19-Cod bones	Thermal treatment	

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Raw Material:



Backbones of salmon (*Salmo salar*) were used as a model of fish transforming industry side-stream.

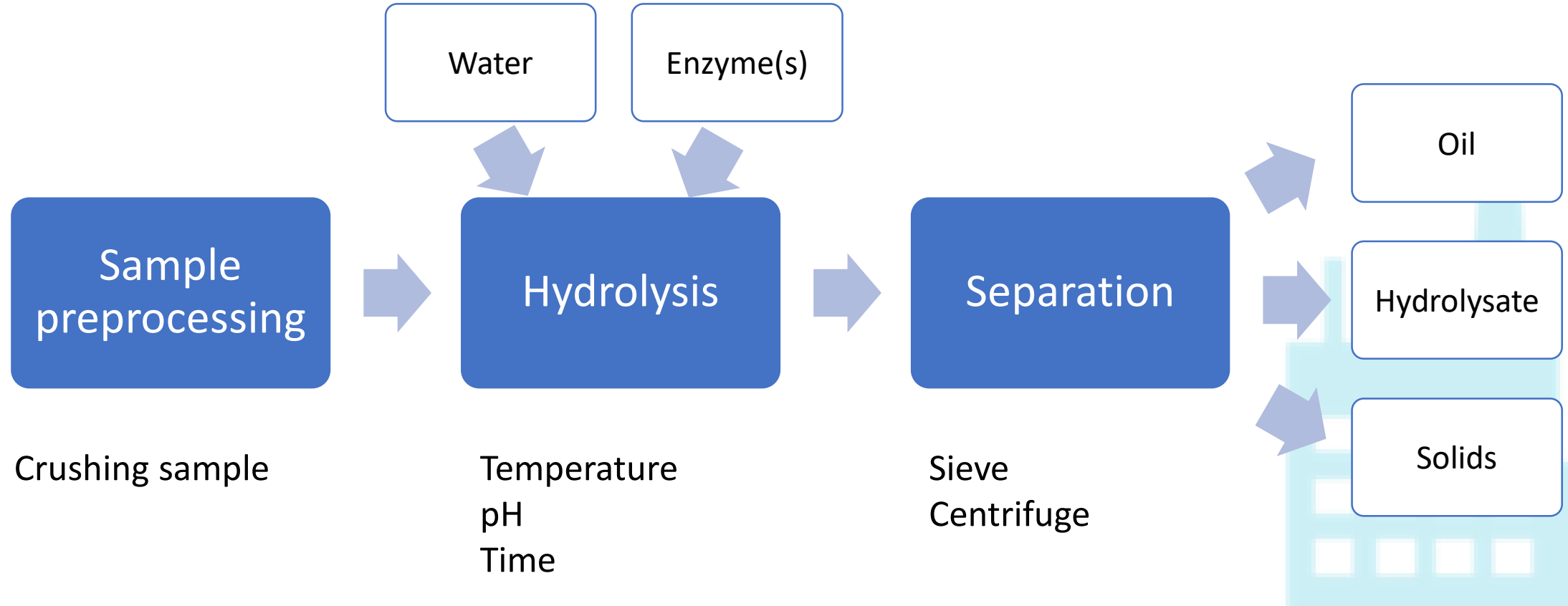
These by-products, along with heads and guts, are used as raw material to produce fishmeal and fish oil.

An improved handling, with the separation of fractions, allowed their use as a food grade fraction unveiling their potential for more valuable uses.



The process:

Enzymatic protein hydrolysis.

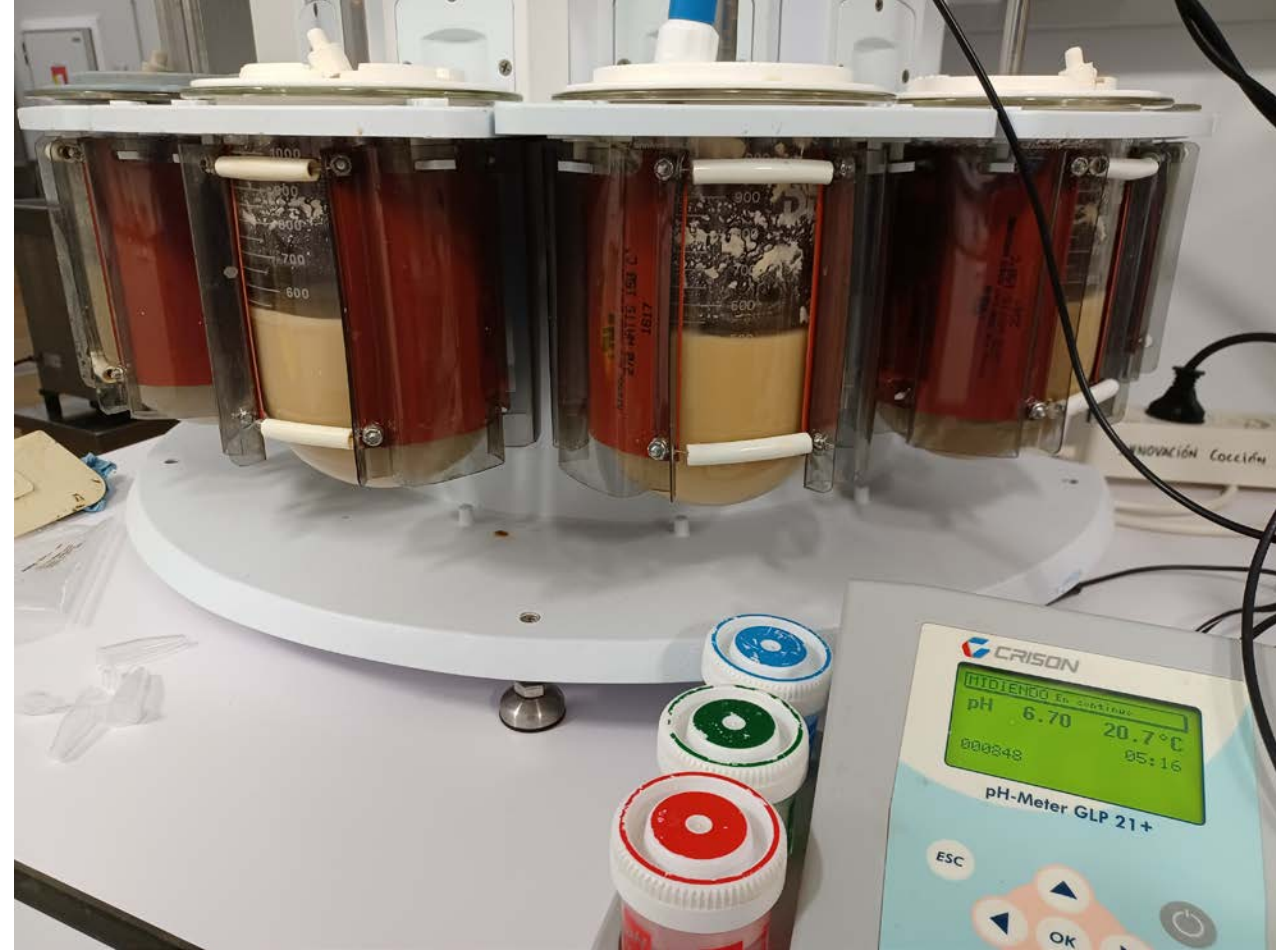


The process:

Enzymatic protein hydrolysis.

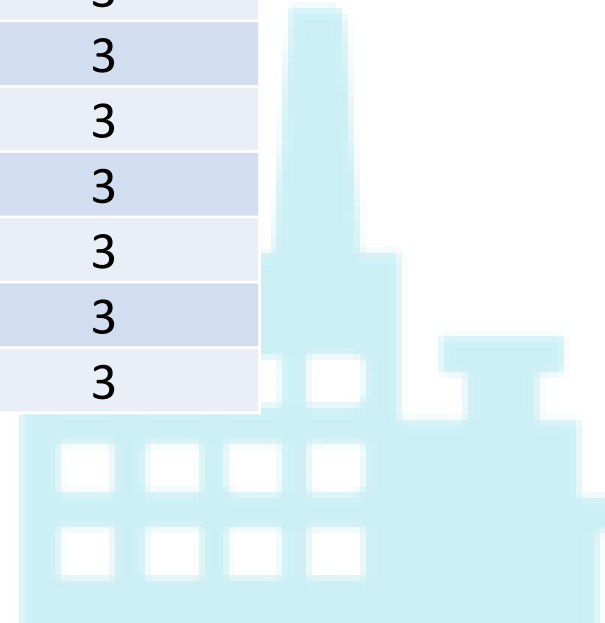
Six enzymes, with different enzymatic activity, were tested to produce protein hydrolysates:

- Broad-spectrum endo-proteases (P)
- Endo-protease of the serine type (A)
- Trypsin specific protease (T)
- Chymotrypsin like protease (C)
- Blend of endo- and exo-peptidases (F)
- Glutamic acid specific protease (G)



The process:

	Enzyme %	S:L	Temp. (°C)	pH	Time (h)
P	1	1:1	50	6	3
A	1	1:1	60	8	3
G	1	1:1	50	6	3
C	1	1:1	70	6	3
T	1	1:1	45	6	3
P+F	1+1	1:1	50	6	3
A+F	1+1	1:1	50	6	3
P+G	1+1	1:1	50	6	3

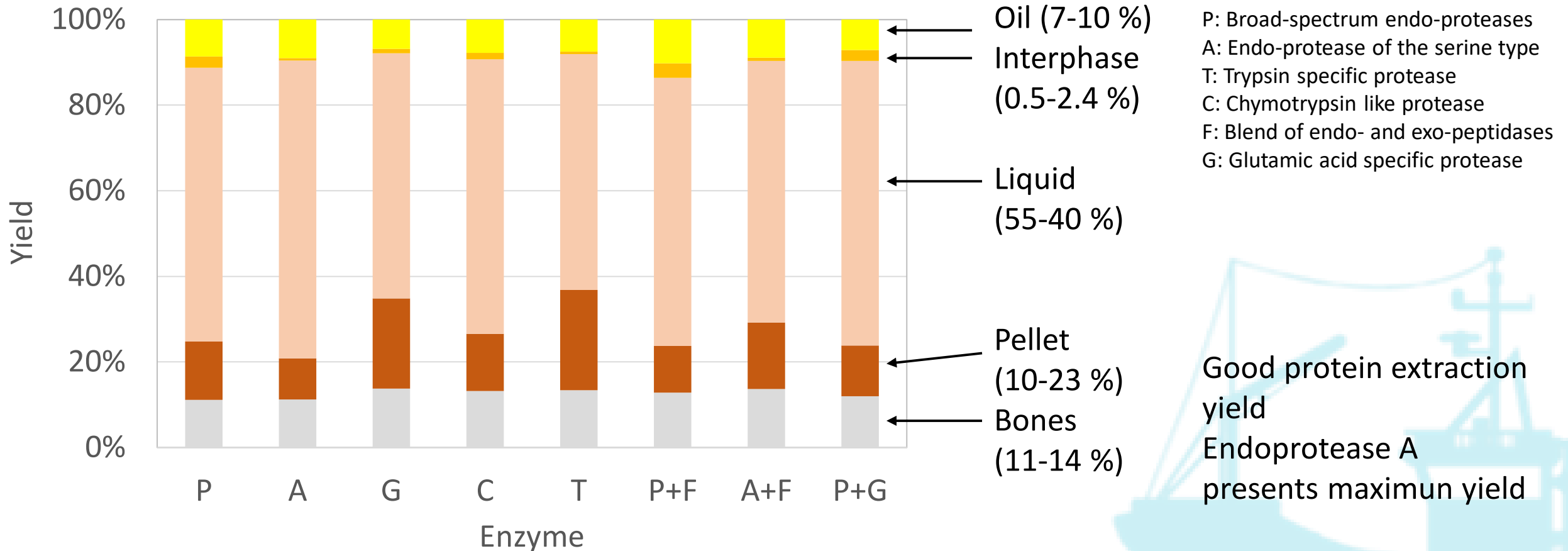


Bioactivity testing

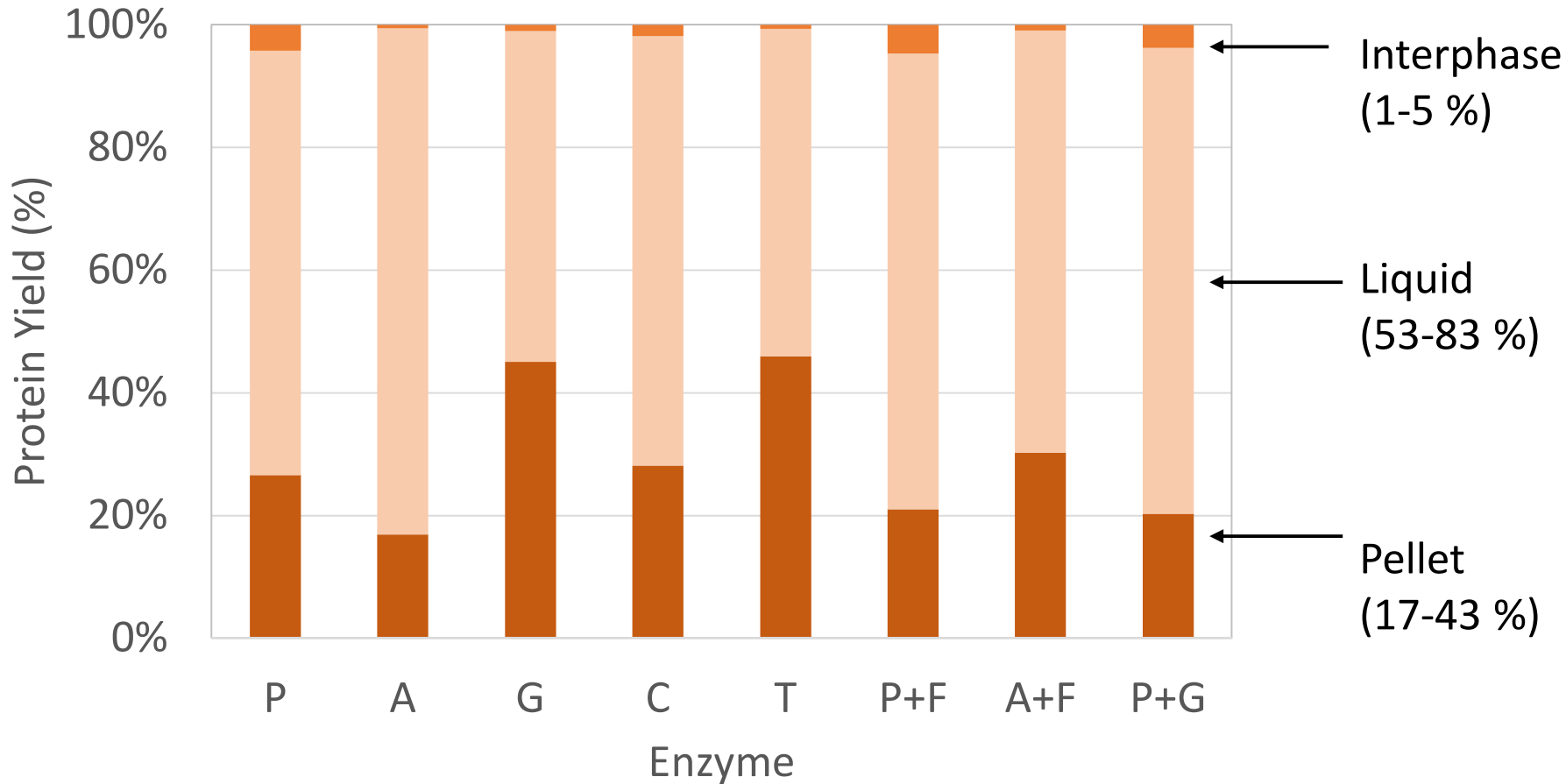
The protein hydrolysates were freeze dried for their evaluation in the bioactivity test.

- Antioxidant activity of the samples were assessed by ABTS method
- Antimicrobial properties were assessed in a two-step approach:
 1. Screening with the agar diffusion method (ADM)
 2. Minimum inhibitory concentration (MIC) analysis of promising samples.
Evaluated against the growth of: *Salmonella enterica* (CECT 4156), *Escherichia coli* (CECT 516), *Bacillus subtilis* (CECT 39), *Bacillus cereus* (CECT 131), *Staphylococcus aureus* (CECT 435), *Aeromonas salmonicida* (CECT 5173) and *Vibrio vulnificus* (CECT 529).
- Antihypertensive capacity was evaluated by the angiotensin converting enzyme (ACE) inhibition method.

Results: Fractions yields



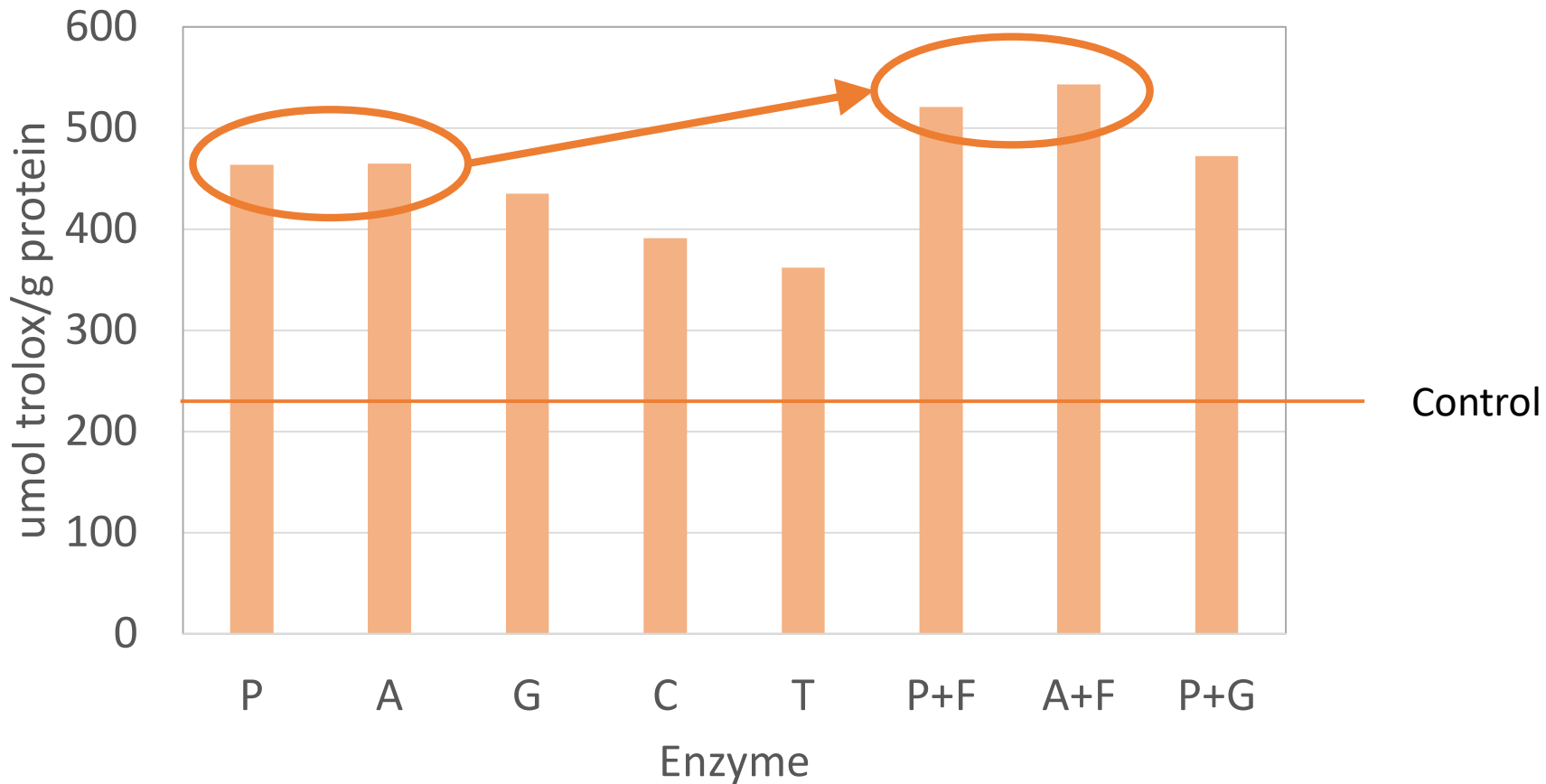
Results: Protein extraction yield



P: Broad-spectrum endo-proteases
 A: Endo-protease of the serine type
 T: Trypsin specific protease
 C: Chymotrypsin like protease
 F: Blend of endo- and exo-peptidases
 G: Glutamic acid specific protease

Maximum protein recovery achieved with the enzyme A.

Results : Antioxidant activity



P: Broad-spectrum endo-proteases
A: Endo-protease of the serine type
T: Trypsin specific protease
C: Chymotrypsin like protease
F: Blend of endo- and exo-peptidases
G: Glutamic acid specific protease

High antioxidant activity.
Exo proteases increases the activity

Results: Antibicrobial capacity

Agar diffusion method

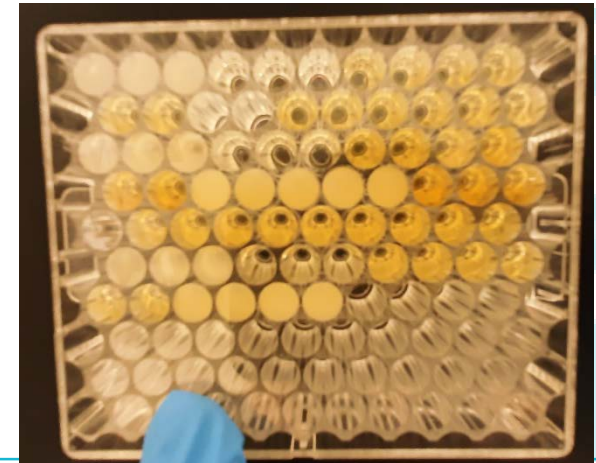
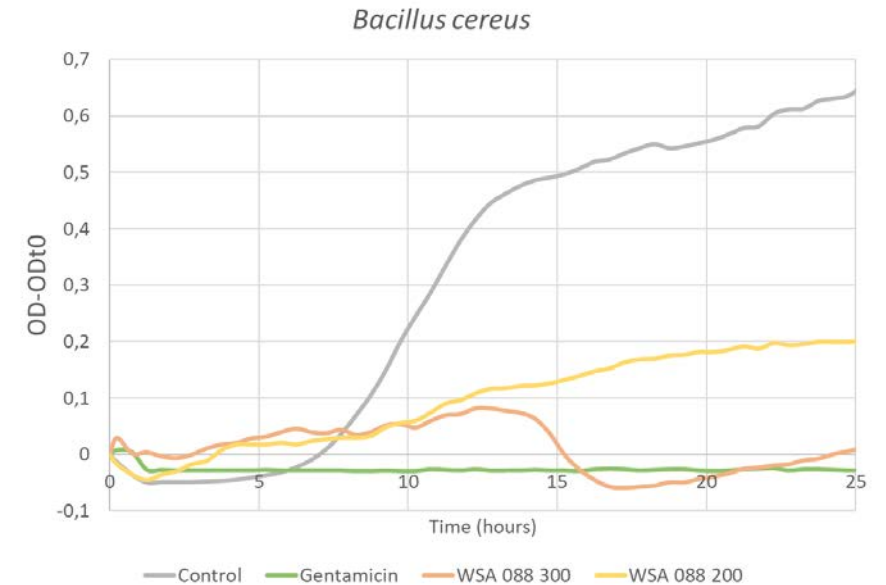
	Salmonella enterica	Escherichia coli	Bacillus subtilis	Bacillus cereus	Staphylococcus aureus	Aeromonas salmonicida	Vibrio vulnificus
P	-	-	-	-	-	-	-
A	-	-	-	-	-	+	-
G	-	-	+	+	+	-	-
C	-	-	-	-	-	-	-
T	-	-	-	-	-	-	-
P+F	-	-	-	-	-	+	-
A+F	-	-	-	-	-	-	-
P+G	-	-	-	-	-	+	-

P: Broad-spectrum endo-proteases
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G: Glutamic acid specific protease



Results: Antibicrobial capacity


Hydrolysate	Strain	MIC (mg/mL)
G	<i>Bacillus subtilis</i> (CECT 39)	250
G	<i>Bacillus cereus</i> (CECT 131)	150
G	<i>Staphylococcus aureus</i> (CECT 435)	ND
A	<i>Aeromonas salmonicida</i> (CECT 5173)	ND
P+F	<i>Aeromonas salmonicida</i> (CECT 5173)	ND
P+G	<i>Aeromonas salmonicida</i> (CECT 5173)	ND



Results: Antihypertensive activity

Antihypertensive activity was determined only in the hydrolysates obtained through the enzymes P, A and G (due to its positive results in ADM test).

	Concentration (mg protein/mL)	ACE Inhibition (%)	IC 50 (mg protein/mL)
P	6.6	95.9	3.1
	1.3	26.9	
A	6.5	100.0	1.9
	1.3	43.9	
G	6.6	96.7	2.7
	1.3	33.1	
P + G	6.8	93.7	2.0
	1.4	44.9	



Promising results, similar to those reported in literature.

Conclusions

🐟 Yields:

- 🐟 Enzymatic process allow to quantitatively extract protein from salmon backbones.
- 🐟 High protein hydrolysate yield have been obtained.

🐟 Antimicrobial:

- 🐟 Few positive results in ADM (with false positives).
- 🐟 Samples with low DH present to higher antimicrobial capacity.
- 🐟 Low MIC values related to analysis with crude hydrolysates.
- 🐟 Sample fractionation might improve results.

🐟 Antioxidant:

- 🐟 High antioxidant values obtained.
- 🐟 Values increased with high DH (use of exo-proteases).

🐟 Antihypertensive

- 🐟 All tested samples showed antihypertensive activity.
- 🐟 IC50 → 1.9-3.1 mg protein /mL.
- 🐟 Values within the reported range (upper limit).
- 🐟 Sample fractionation might improve results.

Thank you! Any questions?

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