Production of small peptides and low molecular weight amino acids by subcritical water from fish meal: Effect of pressurization agent and temperature

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INTRODUCTION

Fish meal: marine food industry by-product

- Aquaculture and pet-food industry
  - ✓ High protein content
  - ✓ Valuable lipid fraction composition
  - ✓ High nutritional protein fraction value
  - ✓ Great amino acid profile
- Improve the use of this by-product
  - ✓ Sustainable exploiting forms

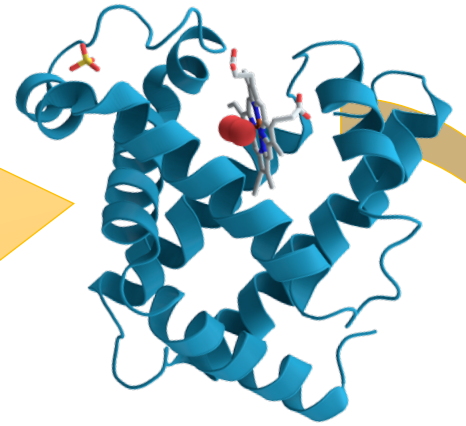
GREEN TECHNOLOGIES

INTRODUCTION

Starting from fish meal as raw material

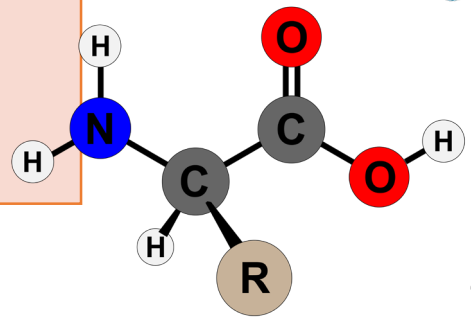
Environmentally friendly processes

- Sustainable methodologies
- Promote green processes to obtain better products
- New functional and healthy products can be obtained



Interesting compounds

- Production of small peptides and free amino acids
- Antioxidant capacity & useful functional properties



INTRODUCTION: Raw material

Tuna fish meal composition

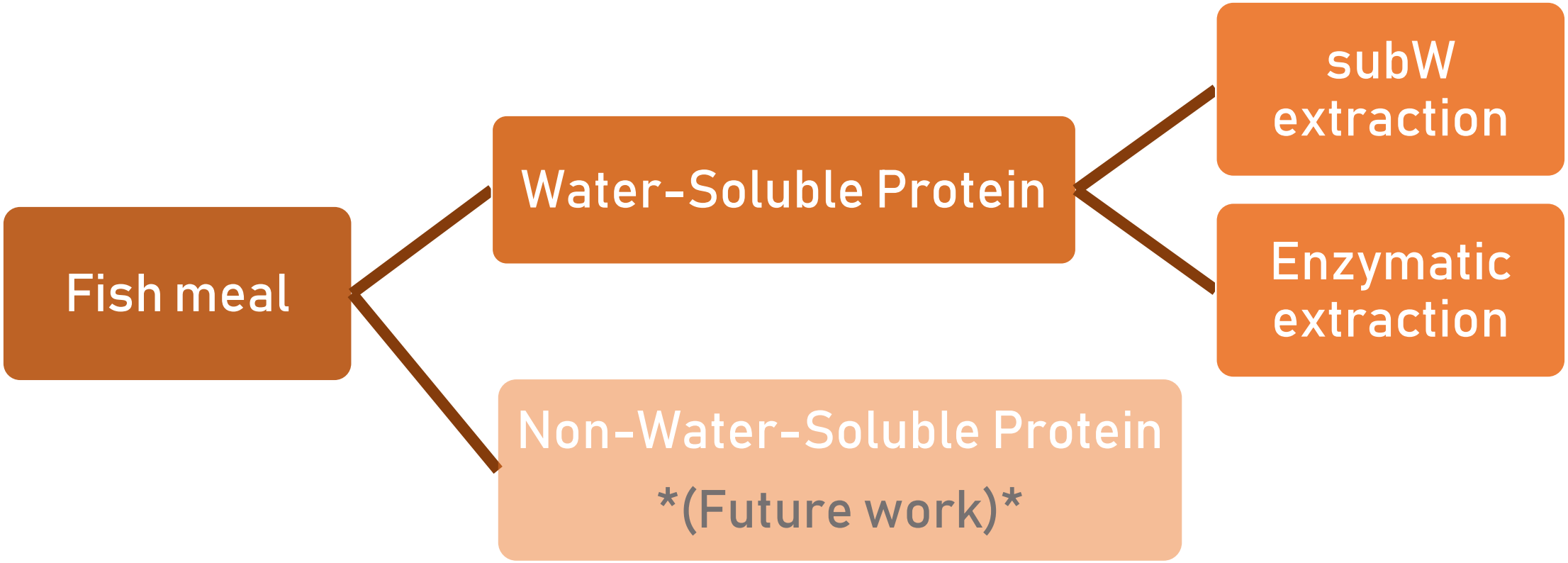
3.4% Moisture	21.7% Ash	6.5% Lipid	51.0% Protein (using N-factor)	Free AA → 5% (95% structural AA)

N-Factor → 5.0 (using %N + AA profile)

10.3% elemental N (CHNS)

↑ AA Profile:
12.7% Glutamic acid
10.8% Aspartic acid
10.3% Alanine
9.9% Glycine

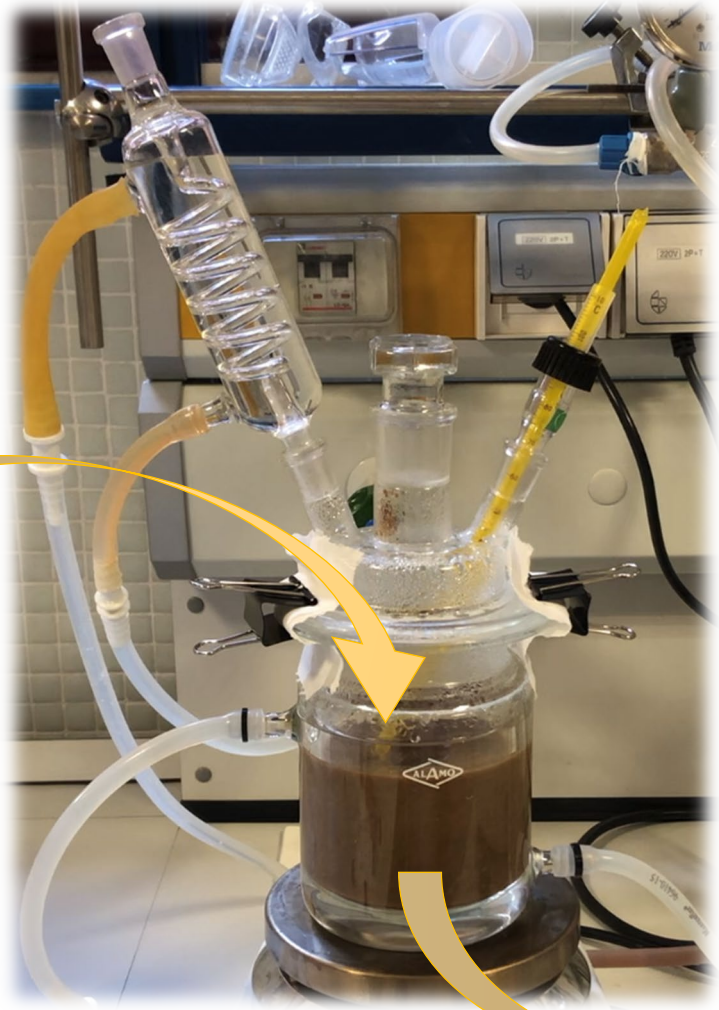
METHODOLOGY



METHODOLOGY

Extraction of WSP

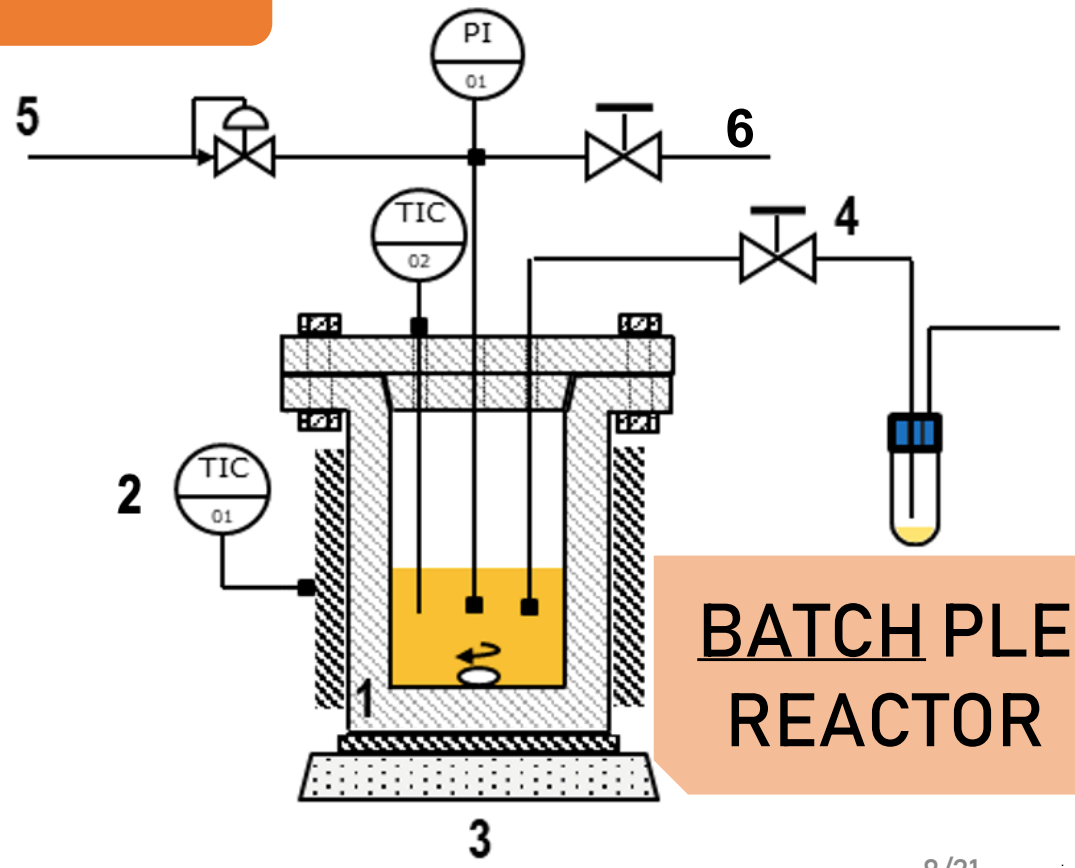
- 4, 8, 16, 24, 48 % fish meal
- 20, 50, 80 °C
- 60 min kinetics



METHODOLOGY

Pressurized Liquid Extractor (PLE)

1. Steel reactor
2. Ceramic resistance for reactor heating
3. Magnetic stirrer
4. Sample valve
5. Gas inlet with pressure regulator
6. Gas outlet via a purge valve



BATCH PLE REACTOR

METHODOLOGY

Pressurized Liquid Extraction (PLE) → Conditions:

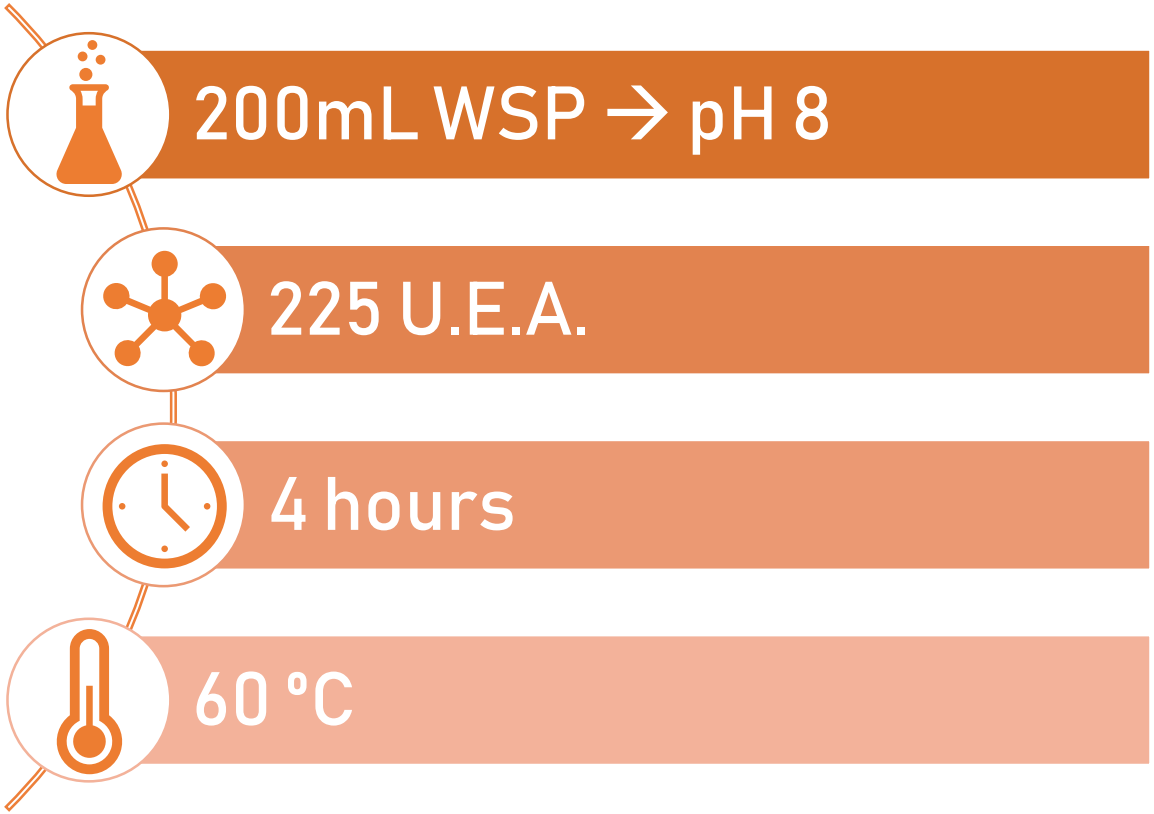
- 200 mL WSP
- 50 bar (5 MPa)
- 5 hours

Pressurization agents	N_2	CO_2	
Temperature	140 °C	160 °C	180 °C

METHODOLOGY

Enzymatic extraction

- Alcalase (*Novozymes®*)
- Novozym (*Novozymes®*)



METHODOLOGY

Analysis of the extracts

- Protein (Lowry)
- Total Amino groups (Ninhydrin)
- Amino Acid profile (GC)
- Peptide size and quantity (SEC)
- Antioxidant power (FRAP/DPPH)
- Surface tension (optical tensiometer)

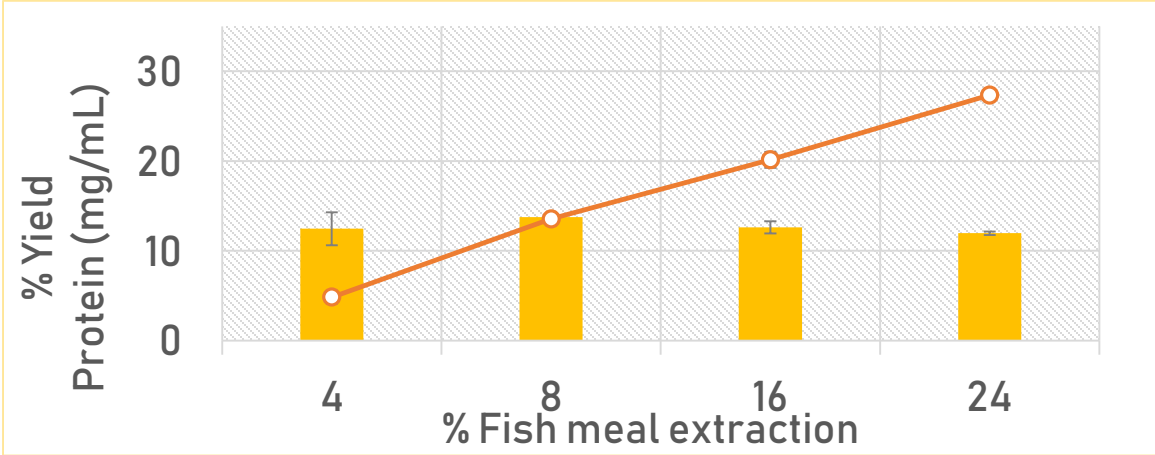
RESULTS → WSP

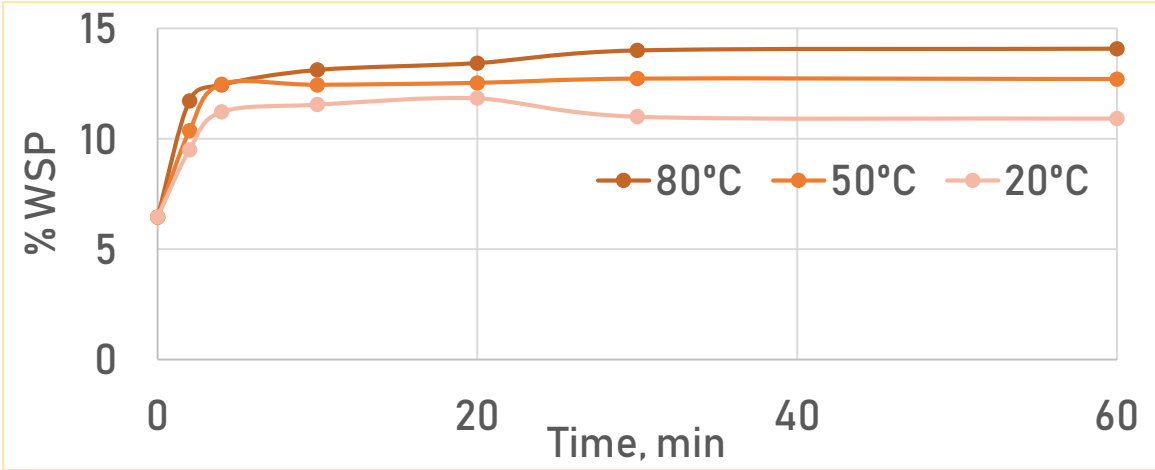
Extraction of WSP

Optimized conditions:

- 16 % fish meal
- 80 ºC
- 30 min

14 % WSP (30% in protein)

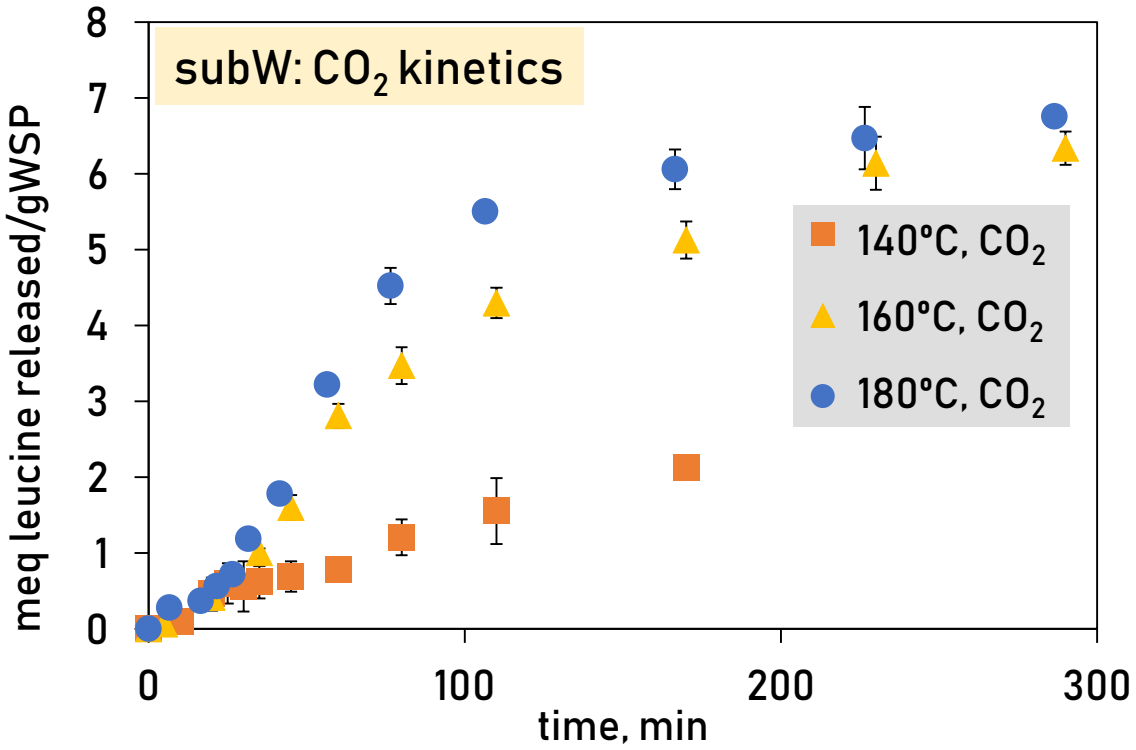


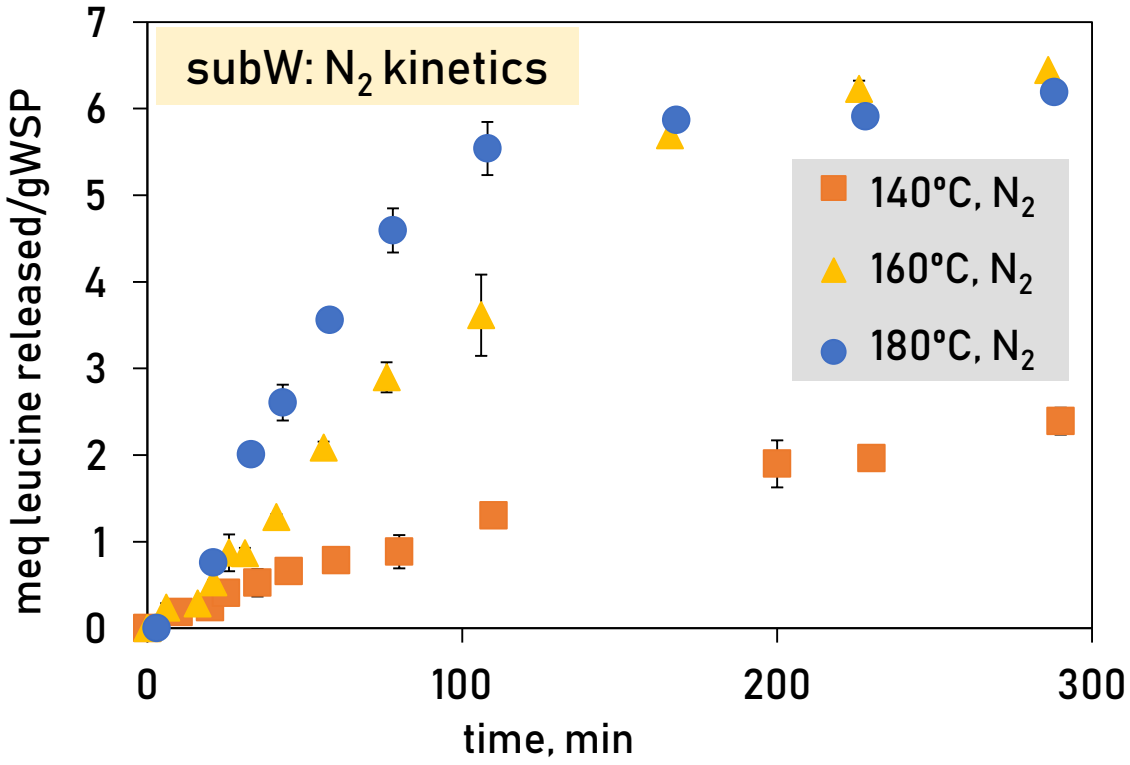


RESULTS → Amino groups

subW > enzymes

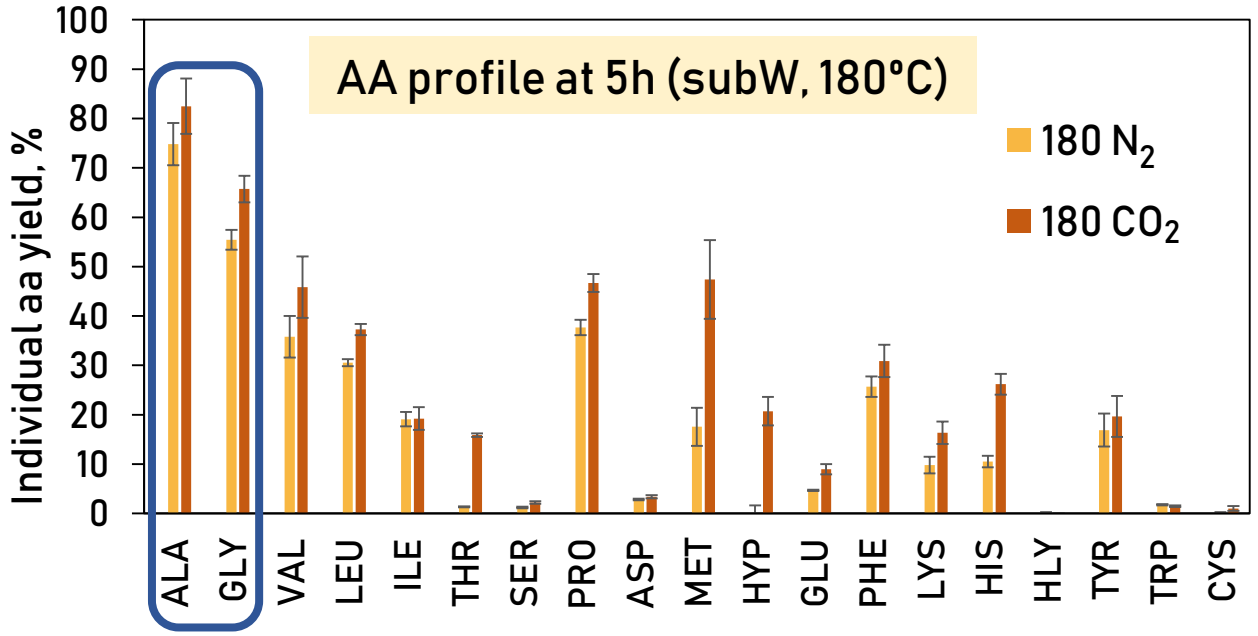
↑Temperature (N_2/CO_2) → ↑SSP / ↑FAA release





RESULTS → Amino Acids

- subW at 180°C + CO_2 → 344 ± 5 mg FAA/g WSP
- subW at 180°C + N_2 → 275 ± 3 mg FAA/g WSP



CO_2 → soluble

$H_2O + CO_2 \rightarrow H_2CO_3 \rightarrow HCO_3^- + H^+$

↓pH solution

Change ionic product

Majority FAA: low Mw

- Gly + Ala → 64% (CO_2)
- Gly + Ala → 59% (N_2)

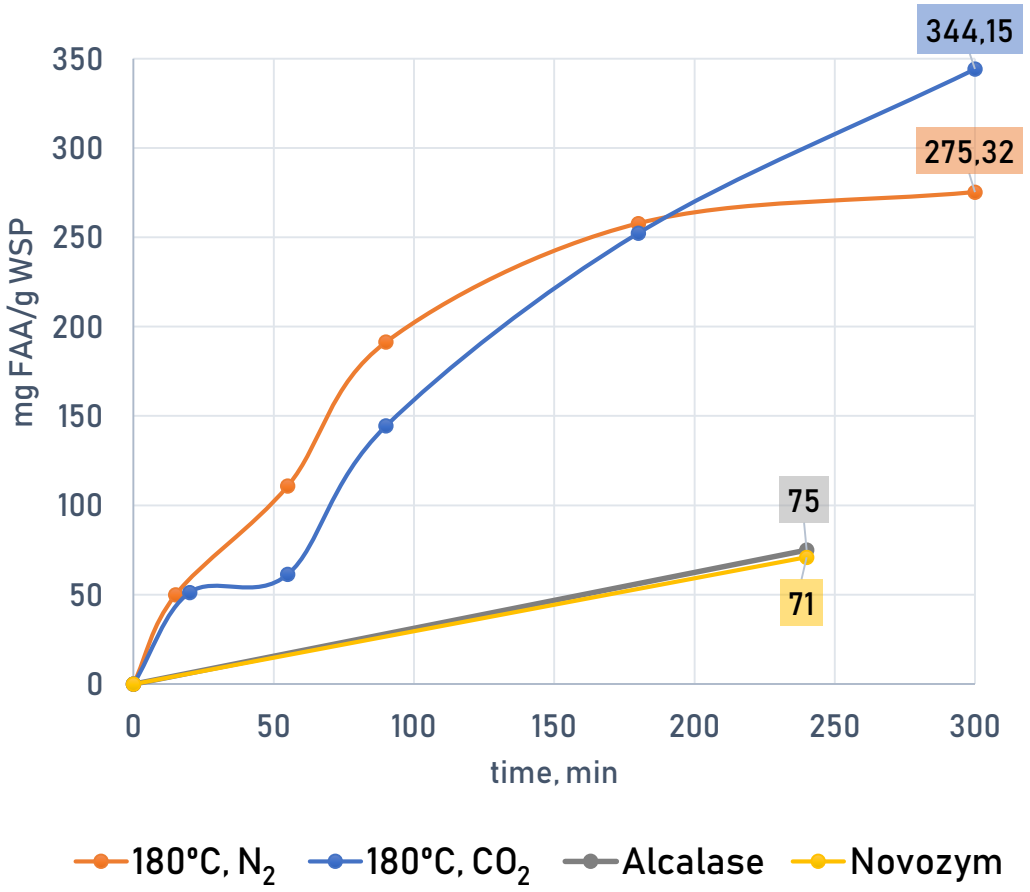
RESULTS → Amino Acids

subW hydrolysis

- ↑ FAA:
  - 180°C, CO_2 → 344 ± 5 mg FAA/g WSP
  - 180°C, N_2 → 275 ± 3 mg FAA/g WSP
- Glycine & Alanine → ↑ FAA yield

Enzymatic hydrolysis

- ↓ FAA:
  - Alcalase → 75 ± 1 mg FAA/g WSP
  - Novozym → 71 ± 0.6 mg FAA/g WSP
- Histidine → ↑ FAA yield



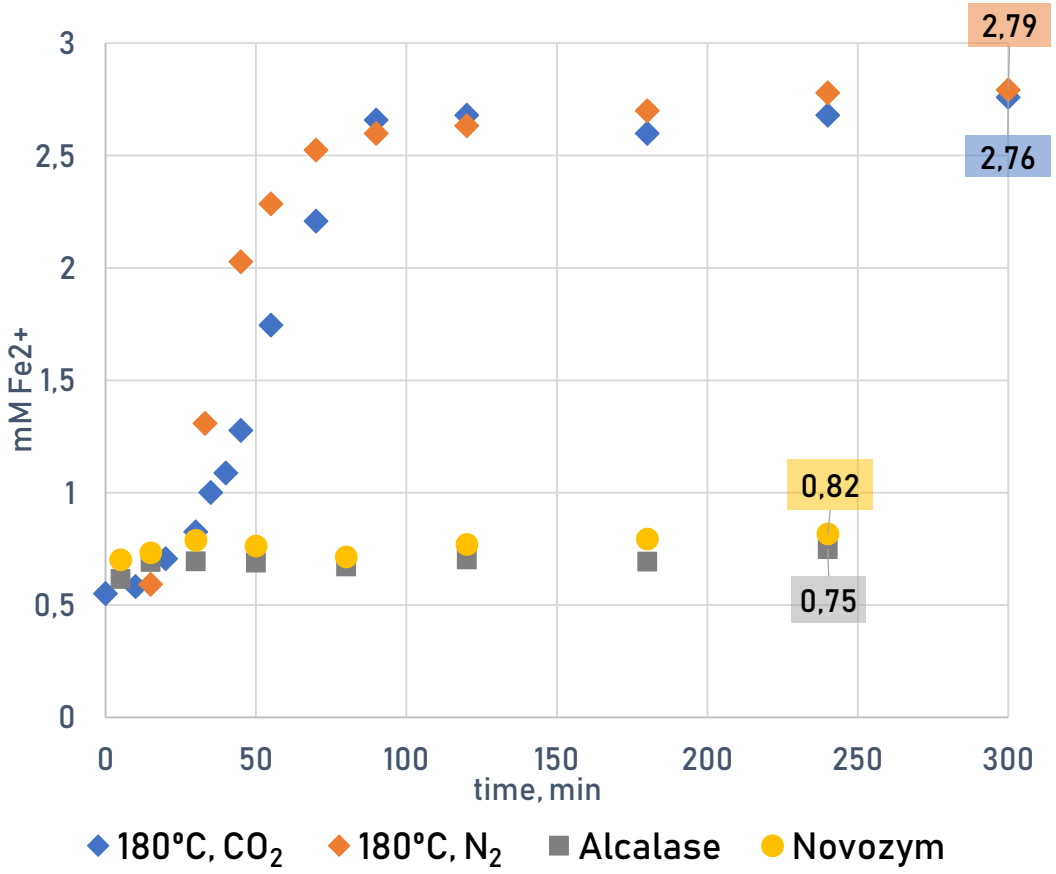
RESULTS → Antioxidant activity

subW hydrolysis

- ↑ Antioxidant capacity:
  - 180°C, CO_2 → 2.76 mM Fe^{2+}
  - 180°C, N_2 → 2.79 mM Fe^{2+}
- ↑ SSP + ↑ FAA = ↑ Antioxidant Capacity

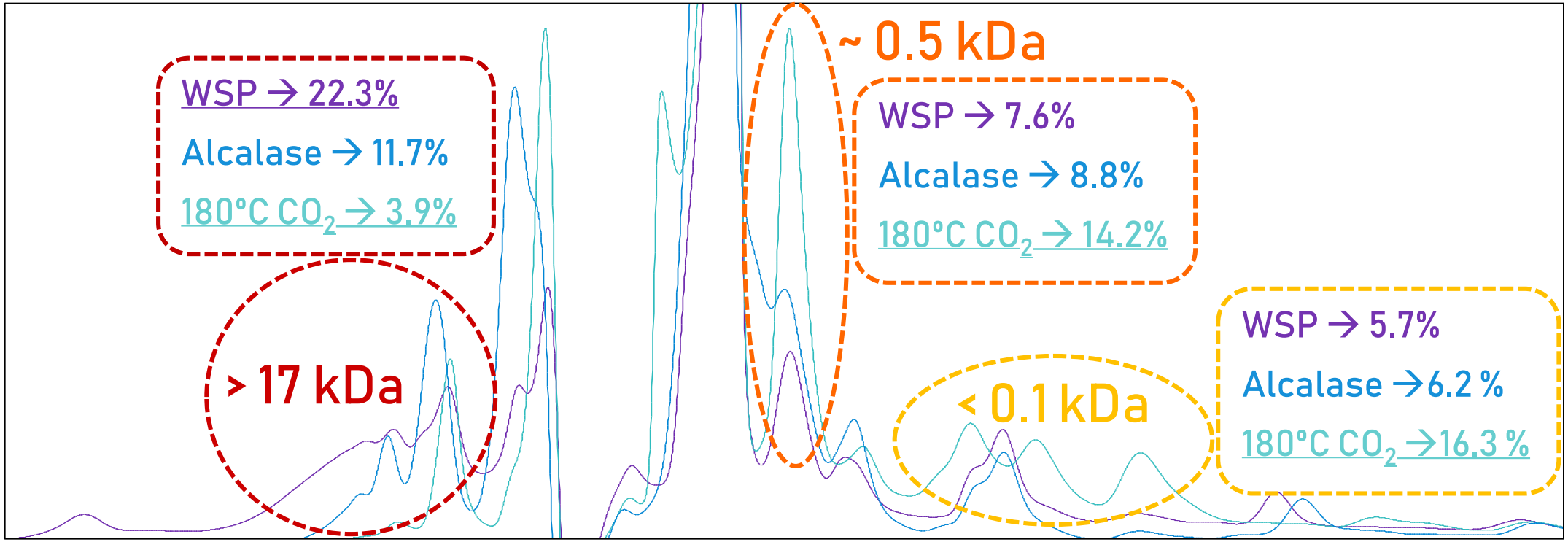
Enzymatic hydrolysis

- ↓ Antioxidant capacity:
  - Alcalase → 0.75 mM Fe^{2+}
  - Novozym → 0.82 mM Fe^{2+}
- ↓ SSP + ↓ FAA = ↓ Antioxidant Capacity



RESULTS → Peptide size

- Size Exclusion Chromatography (SEC)



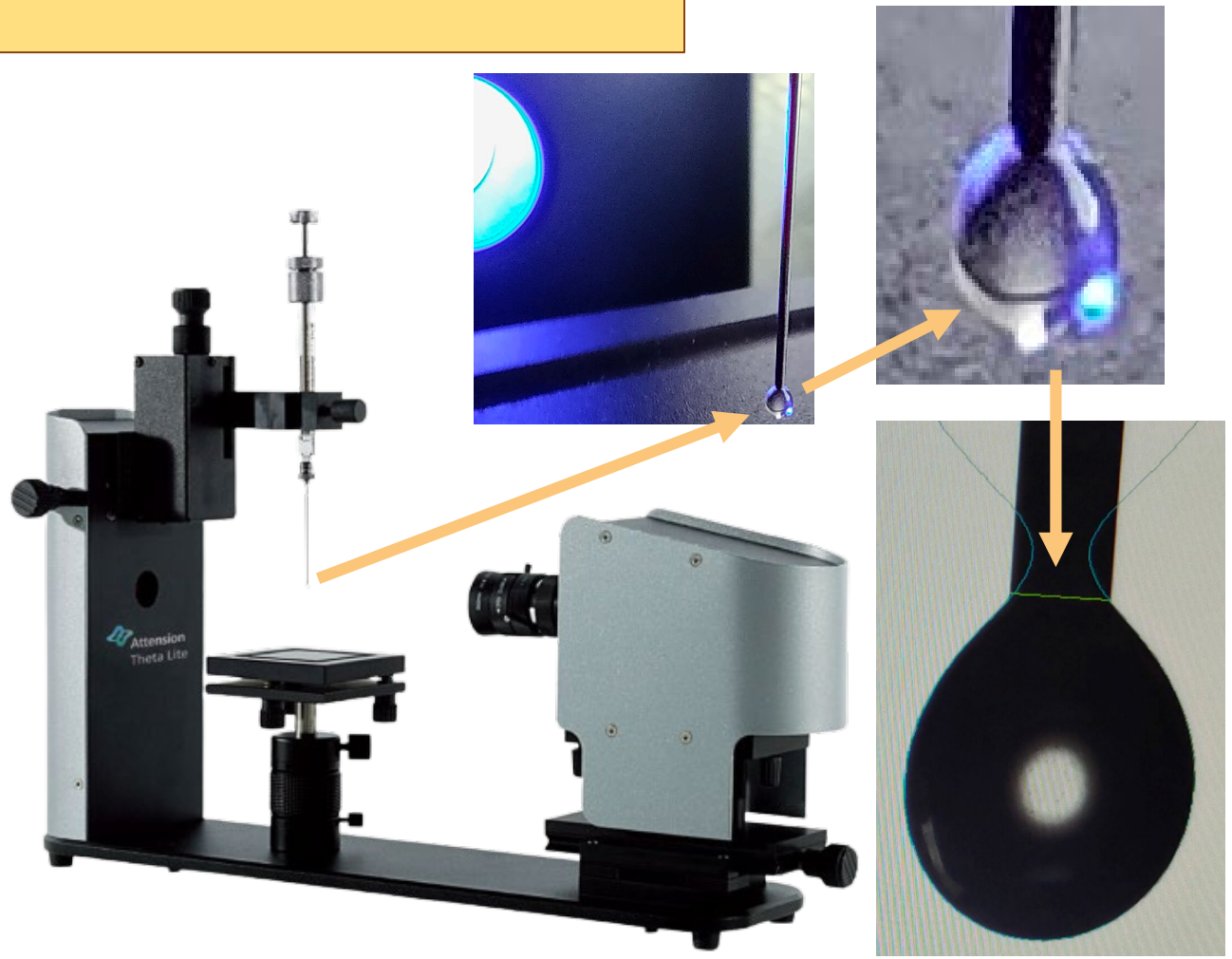
RESULTS → Surface tension

	S.T. [mN/m]
Water	72.5 ± 0.7
WSP	49.3 ± 0.5
Alcalase	56.1 ± 0.9
180°C CO_2	54.5 ± 0.5

Extracts decrease water S.T.

↓ S.T. → ↑ Functional properties

Emulsifying	Foaming



CONCLUSIONS

- subW is a green technology more effective than enzymes
- Higher temperature (subW) → Higher protein hydrolysis
- Effectiveness increased pressurizing with CO_2
- Higher protein hydrolysis → Higher reducing capacity
- SEC confirms better hydrolysis → subW
- Fish WSP + subW → ↑↑ Functional properties

FUTURE WORK

Analyse more functional properties

Non-Water-Soluble Protein (N-WSP)

Compare results with vegetal by-products

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Thanks for your attention

Acknowledgements

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