

Production of Enzymatic Hydrolysates From Blue Shark Skin By-product Using a Biorefinery Approach

Ezequiel R. Coscueta^{1,*}, María Emilia Brassesco¹, Ana Rosa^{2,3}, André Almeida^{2,3} & Manuela Pintado¹

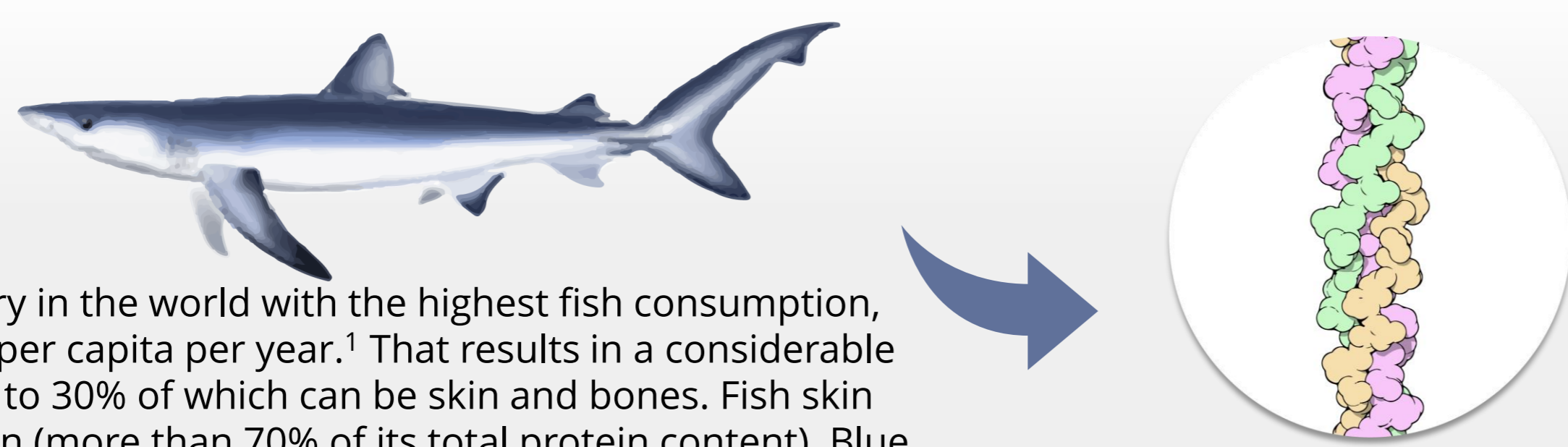
¹ Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal. ² SEBOL, Comércio e Indústria do Sebo, S.A., Grupo ETSA, Santo Antão do Tojal, Portugal. ³ ITS, Indústria Transformadora de Subprodutos, S.A., Grupo ETSA, São José da Lamasosa, Portugal.



Introduction

Blue Shark Skin

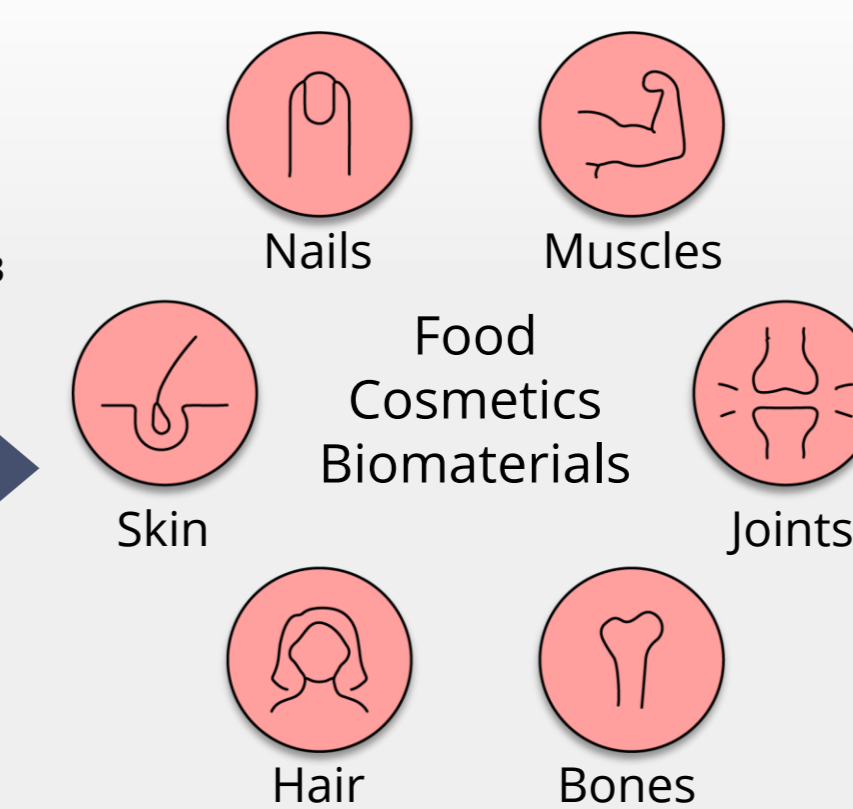
Portugal is the 3rd country in the world with the highest fish consumption, with an average of 59 kg per capita per year.¹ That results in a considerable amount of fish waste, up to 30% of which can be skin and bones. Fish skin mainly comprises collagen (more than 70% of its total protein content). Blue shark skin is a by-product of the fishing industry that is rich in collagen.²



Collagen Type I

Collagen is a structural protein found in animal extracellular matrices and has a wide range of commercial applications. In the past, bovine and porcine sources have been the primary sources of collagen, but due to religious beliefs and some infectious diseases, other sources are in debate.

Enzymatic Hydrolysis³



High-value Ingredient
Marine-based collagen is an attractive alternative for the industry.⁴ This study used a biorefinery approach to valorize blue shark skin waste producing low molecular weight peptides.

Methods and Results

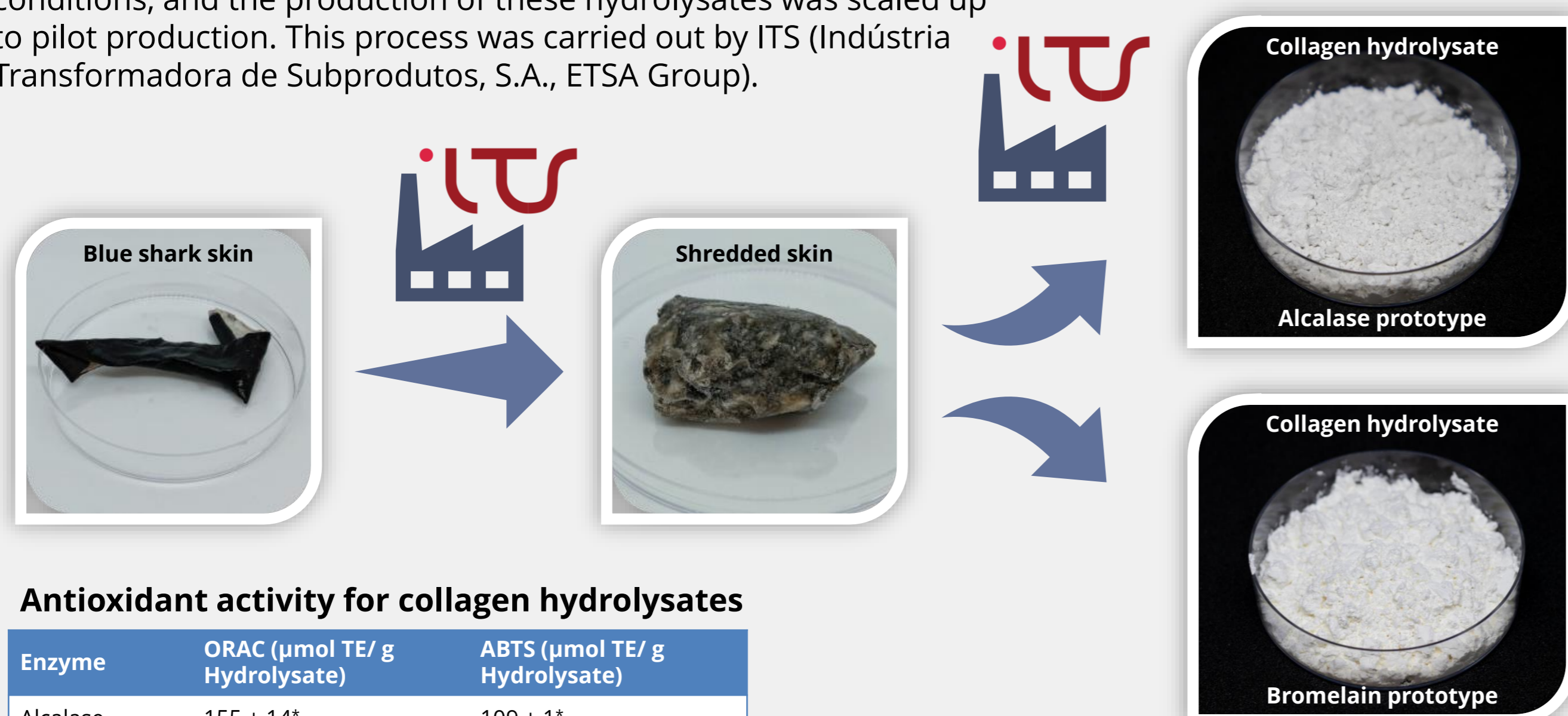
applied sciences
Collagen-Based Bioactive Bromelain Hydrolysate from Salt-Cured Cod Skin
Ezequiel R. Coscueta¹, María Emilia Brassesco¹ and Manuela Pintado¹

Process

We followed a process reported by our group using another type of marine matrix (cod skin) to produce collagenic peptide hydrolysates and non-collagenic protein hydrolysates.³

Scale Up

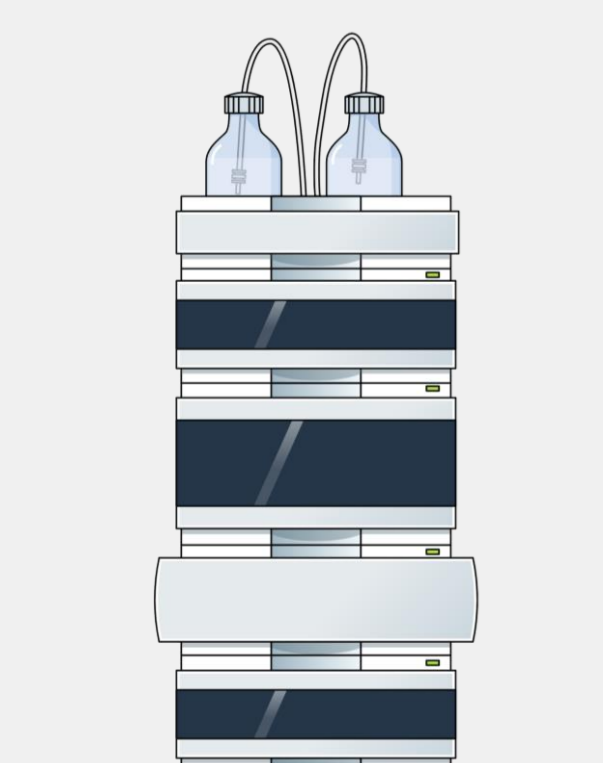
Once we validated the predictive models, we chose the most viable conditions, and the production of these hydrolysates was scaled up to pilot production. This process was carried out by ITS (Indústria Transformadora de Subprodutos, S.A., ETSA Group).



Antioxidant activity for collagen hydrolysates

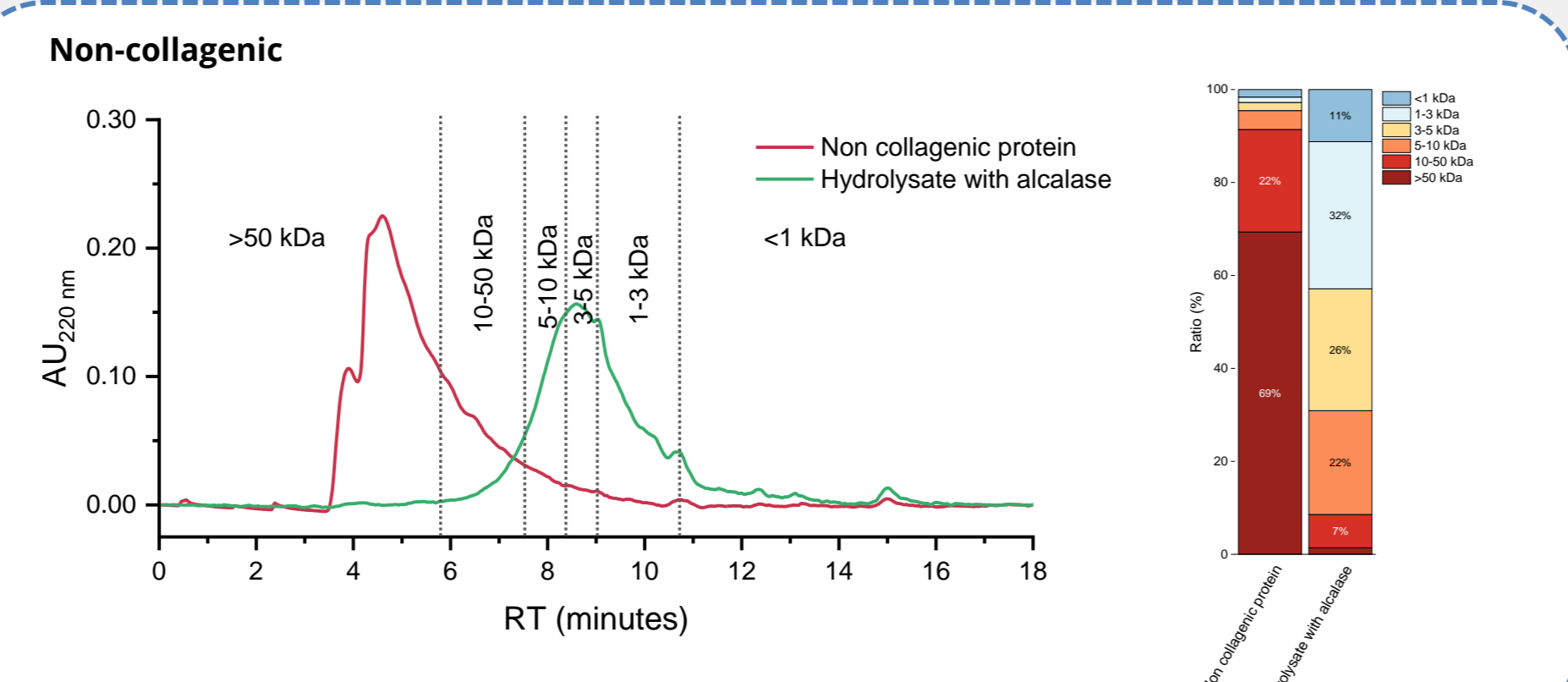
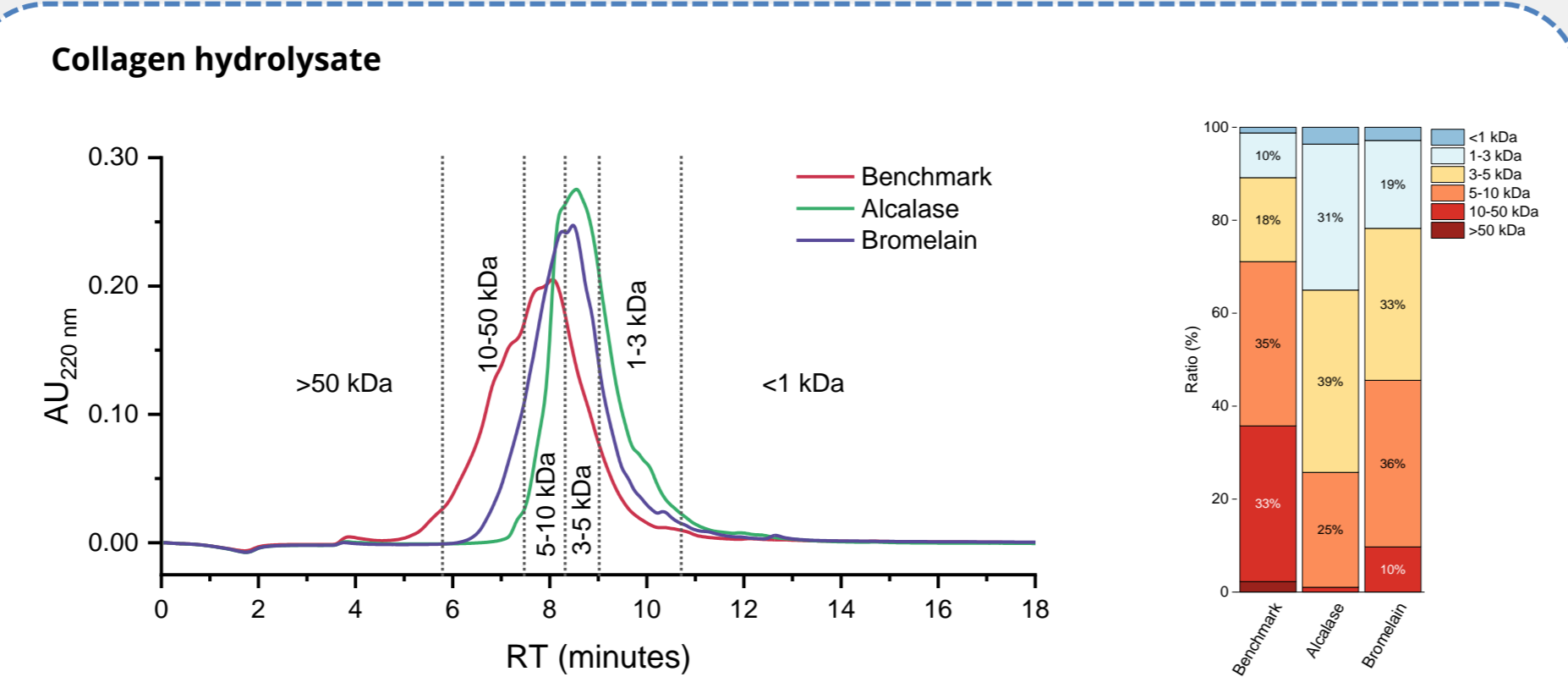
Enzyme	ORAC (μmol TE/g Hydrolysate)	ABTS (μmol TE/g Hydrolysate)
Alcalase	155 ± 14*	109 ± 1*
Bromelain	196 ± 5*	48 ± 3*

*Values in the same column are different for a 5% probability level (T-test).



Peptide profile
HPLC coupled with Size Exclusion Chromatography (HPSEC)

For the scaling up of the hydrolysate of non-collagenic proteins, only alcalase was selected. Further results of this ingredient are not shown since they are part of a study for potential application in aquaculture diets. This non-collagenic fraction presents part of the strong fish aromas, which makes it interesting for this field of application.



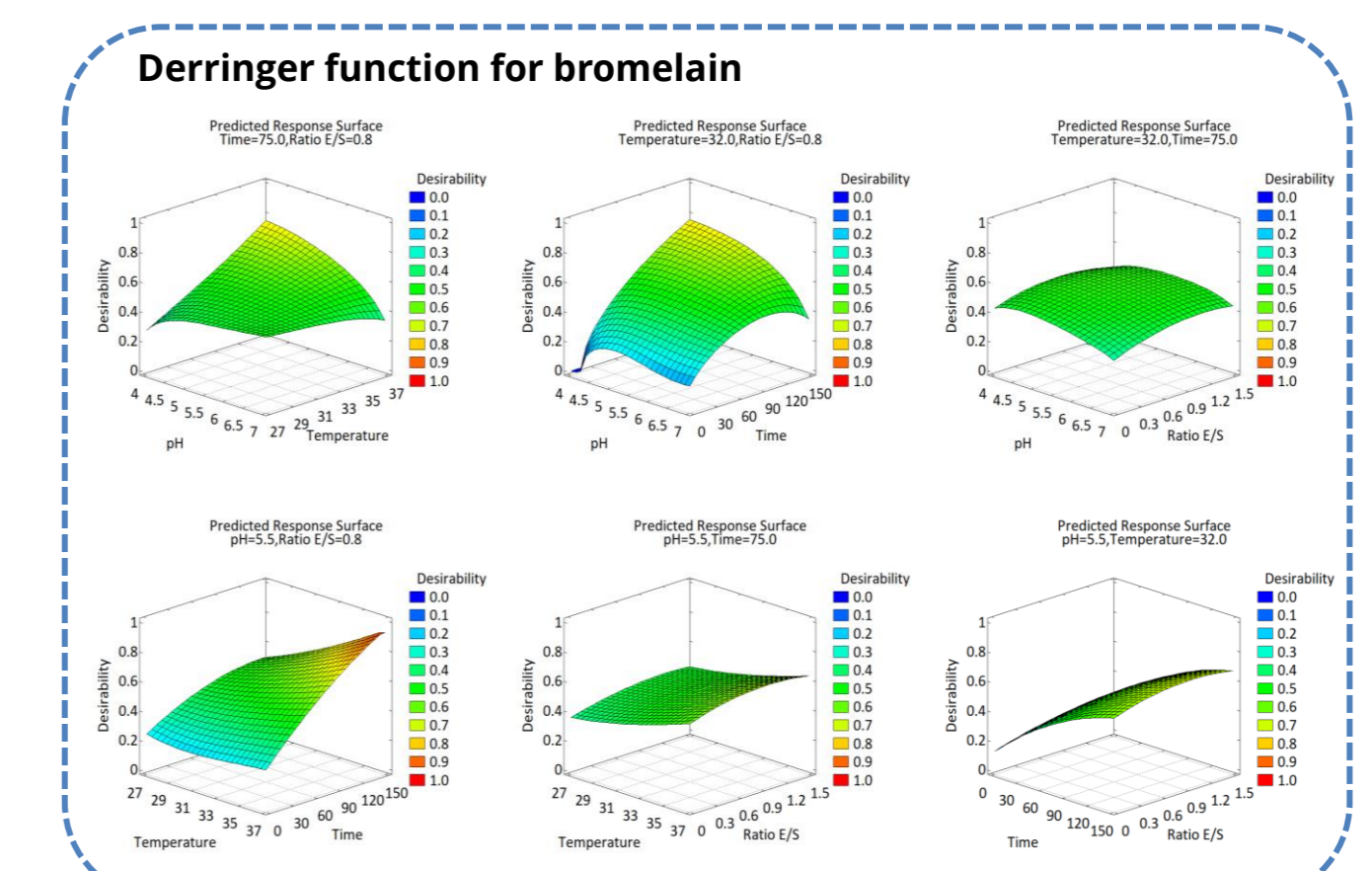
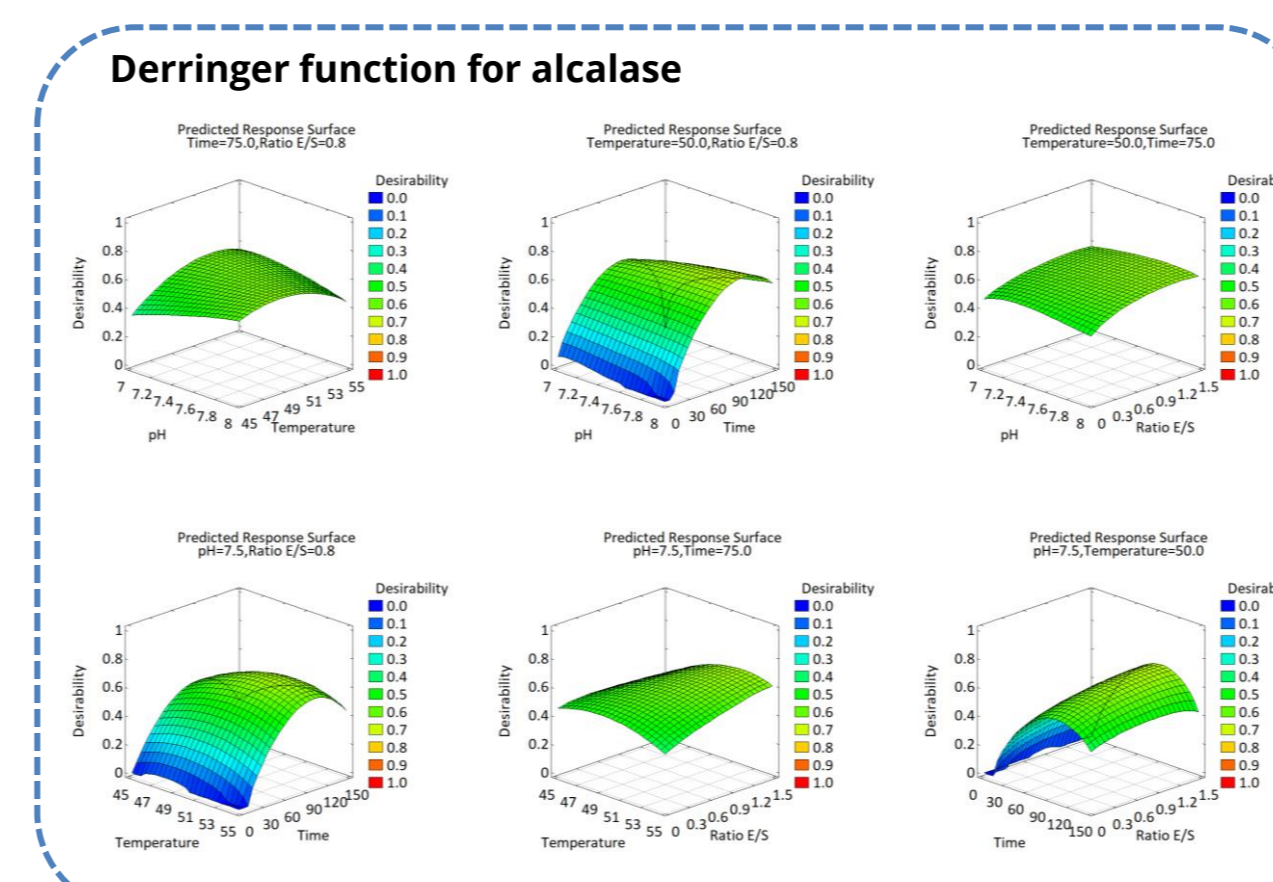
Optimization

We performed a multifactorial optimization study for both matrices with two enzymes. We evaluated two proteases: alcalase (of microbial origin) and bromelain (of vegetable origin). The responses were the degree of hydrolysis (DH) and antioxidant activity (ORAC) for all models and, additionally, protein solubilization for the case of collagen hydrolysates.

Collagen hydrolysates

Factor	Alcalase		Bromelain		Units
	Low	High	Low	High	
A: pH	7.0	8.0	4.0	7.0	
B: Temperature	45.0	55.0	27.0	37.0	°C
C: Time	15	135	15	135	min
D: Ratio E/S	0.1	1.5	0.1	1.5	%

Response	Units
ORAC	μmol TE/g Protein in skin (DB)
Protein (BCA)	Mg BSA/g Protein in skin (DB)
DH (TNBS)	%



Factor	Optimum	
	Alcalase	Bromelain
pH	7.7	4.0
Temperature (°C)	51.8	36.9
Time (min)	103	135
Ratio E/S (%)	1.5	0.5

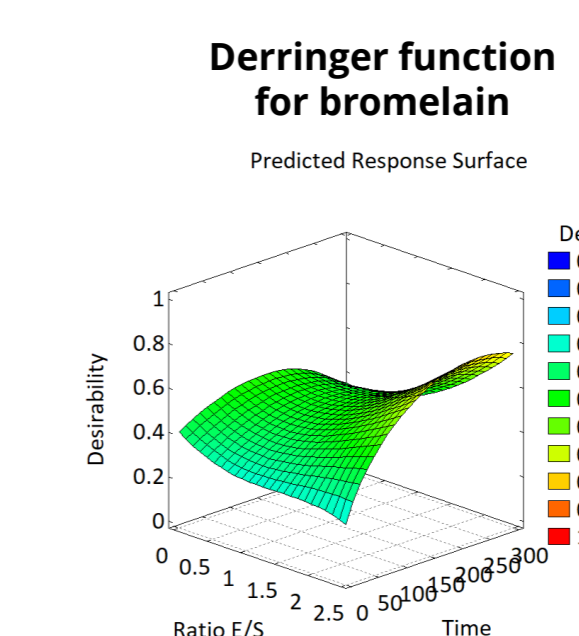
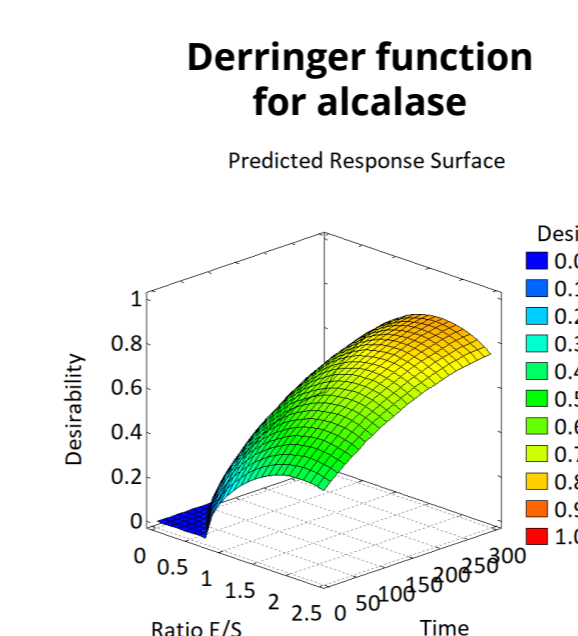
Response	Optimum	
	Alcalase	Bromelain
ORAC (μmol TE/g Protein in skin)	28.1	5.5
Protein (mg BSA/g Protein in skin)	110.0	36.3
DH (%)	13.2	1 approx.

In the conditions tested closer to the optimum we had a significantly different yield in the digestion of the initial mass of skin. Considering the complete process (pre-treatment followed by hydrolysis) for alcalase there is 13±3% remaining mass (dry basis) and for bromelain 35±5% (dry basis). It is important to consider that the skin has 75.6% moisture and 86.06% protein on a dry basis. In addition, in the pre-treatment, the non-collagenous proteins extracted correspond to 31.2% of the total protein content of the skin.

Non-collagenic hydrolysates

Factor	Alcalase		Bromelain		Units
	Low	High	Low	High	
A: Ratio E/S	0.5	2.5	0.5	2.5	% WB
B: Time	30	270	30	270	min

Response	Units
ORAC	μM TE
DH (TNBS)	%



Factor	Optimum	
	Alcalase	Bromelain
Ratio E/S (%)	1.8	2.5
Time (min)	270	237

Response	Optimum	
	Alcalase	Bromelain
ORAC (μM TE)	971.7	669.8
DH (%)	31.8	9.0

Conclusions

We successfully developed a sustainable process to obtain low molecular weight hydrolysates with antioxidant activity. In all cases, alcalase was the enzyme with the best performance. The optimized hydrolysates showed molecular profiles, bioactivities, and organoleptic characteristics of interest for the nutraceutical and cosmetic industries (in the case of collagen hydrolysates) and aquaculture (in the case of non-collagenic hydrolysates).

References

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Acknowledgements

This work is co-funded by the European Regional Development Fund (FEDER), through the Competitiveness and Internationalisation Operational Programme (POCI), within the scope of the **FISHCOLBOOSTER**: Development of fish collagen peptides in integrated system with obtaining fractions of high value for human food, aquaculture and cosmetics. (POCI-01-0247_FEDER-49636).