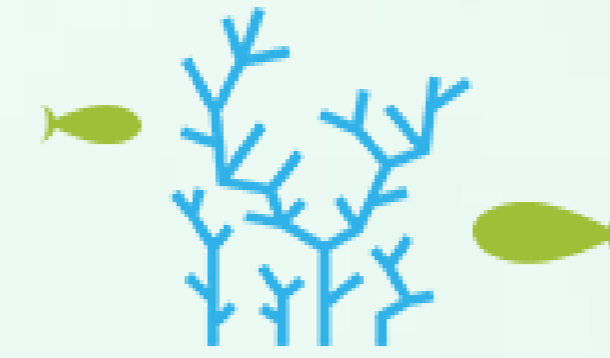


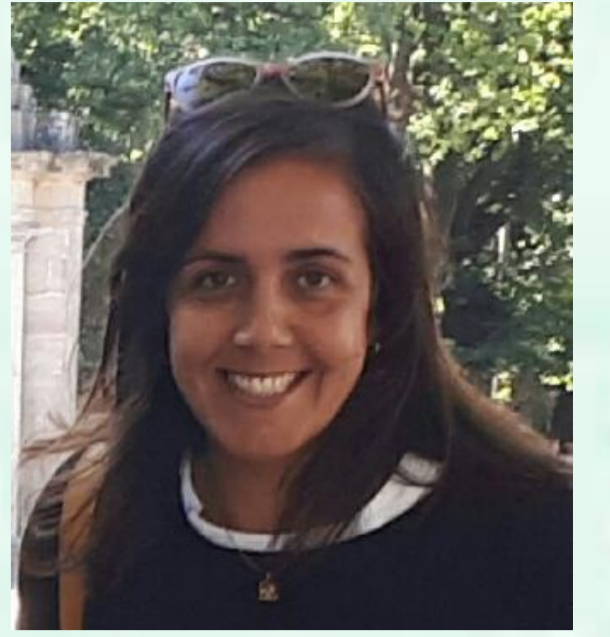
SCYLORHINUS CANICULA SKIN: OPTIMIZATION OF COLLAGEN EXTRACTION USING RESPONSE SURFACE METHODOLOGY



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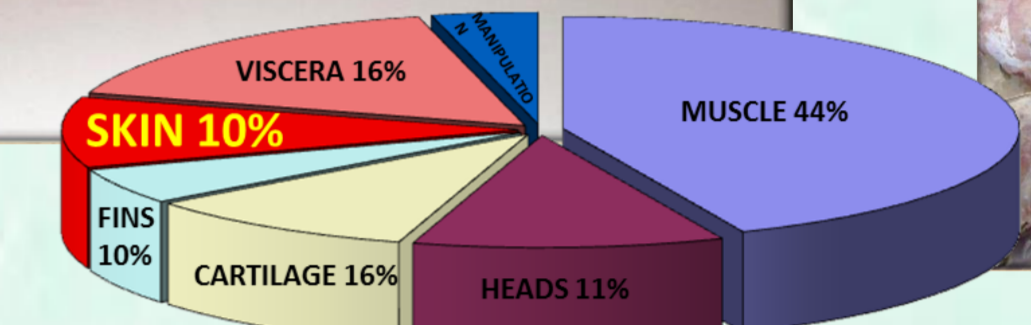
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INTRODUCTION AND OBJECTIVE

- **Small-spotted catshark** is one of the most abundant elasmobranchs in the Northeast Atlantic Sea. Although landings are made for human consumption (rendering 10% of fish weight in the form of **skin by-products**), it generally has a **low commercial value** and is taken as a bycatch, with very **high discard rates** reaching 100% in some European fisheries.
- The reduction of post-harvest fish losses (discards and by-products) by the promotion of a **full use of fishing captures** is one of the main purposes of UE fishing policies.
- As collagens obtained from marine sources are applied increasingly as **alternatives to mammalian collagens** for cosmetics, tissue engineering and other biomedical and pharmaceutical uses, due to safety reasons and ethical or religious constraints, **fish skins** represent an excellent and abundant source for obtaining this biomolecule.

- The **aim of the study** was to analyze the influence of solvent concentration, temperature and extraction time on the extractability of collagen from Small-spotted catshark (*Scyliorhinus canicula*) skin.



EXPERIMENTAL WORK

Sampling

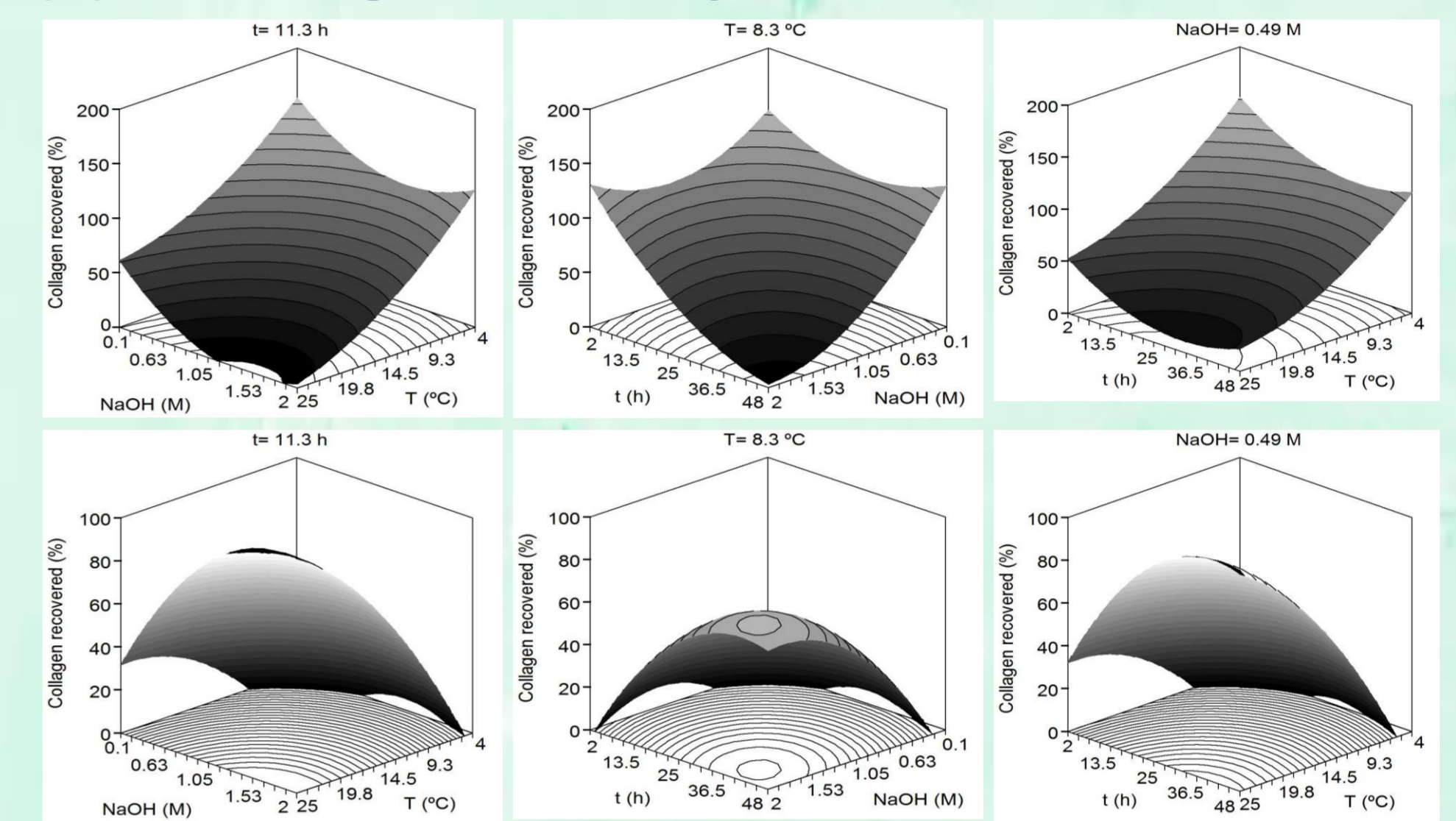


First experimental design: NaOH extraction

Experiment number	T (°C)	NaOH (M)	t (h)	T cod	NaOH cod	t cod
1	8.26	0.49	11.33	-1.000	-1.000	-1.000
2	20.74	0.49	11.33	1.000	-1.000	-1.000
3	8.26	1.61	11.33	-1.000	1.000	-1.000
4	20.74	1.61	11.33	1.000	1.000	-1.000
5	8.26	0.49	38.67	-1.000	-1.000	1.000
6	20.74	0.49	38.67	-1.000	-1.000	1.000
7	8.26	1.61	38.67	-1.000	1.000	1.000
8	20.74	1.61	38.67	1.000	1.000	1.000
9	4.00	1.05	25.00	-1.682	0.000	0.000
10	25.00	1.05	25.00	1.682	0.000	0.000
11	14.50	0.10	25.00	0.000	-1.682	0.000
12	14.50	2.00	25.00	0.000	1.682	0.000
13	14.50	1.05	2.00	0.000	0.000	-1.682
14	14.50	1.05	48.00	0.000	0.000	1.682
15	14.50	1.05	25.00	0.000	0.000	0.000
16	14.50	1.05	25.00	0.000	0.000	0.000
17	14.50	1.05	25.00	0.000	0.000	0.000
18	14.50	1.05	25.00	0.000	0.000	0.000
19	14.50	1.05	25.00	0.000	0.000	0.000
20	14.50	1.05	25.00	0.000	0.000	0.000

NaOH Optimum Conditions:
8,3°C / 11h / 0,49N NaOH

Combined effect of NaOH (M), time (t) and temperature (T) on collagen recovery from *S. canicula* skins.



Sampling



NaOH extraction

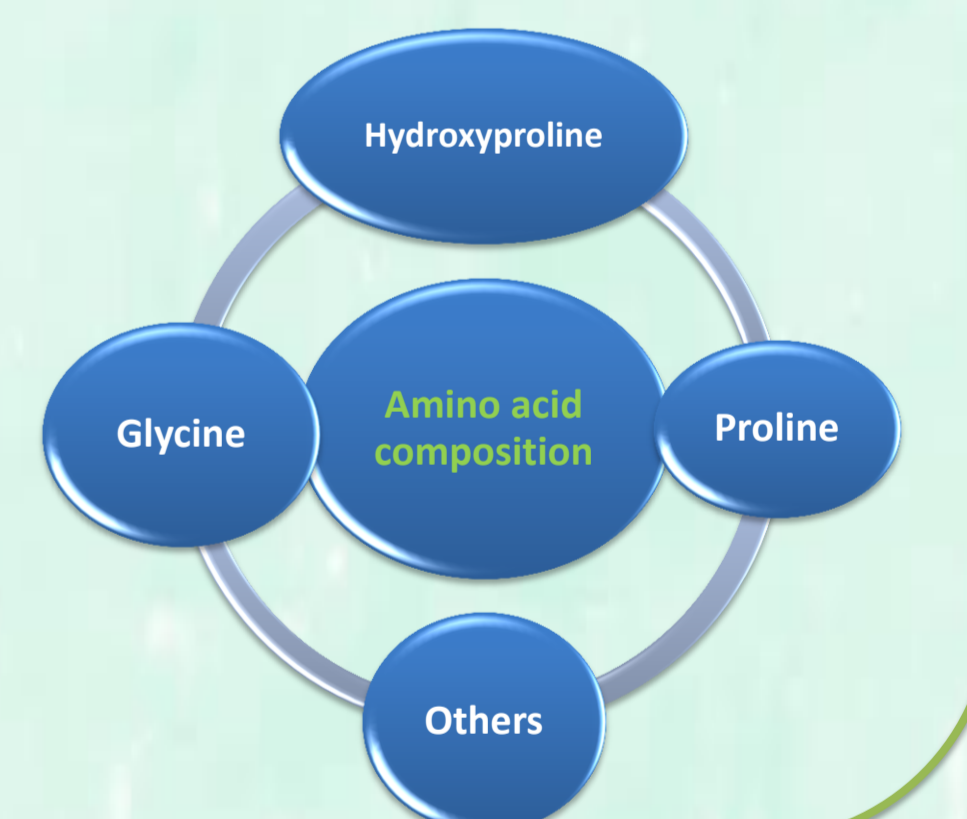
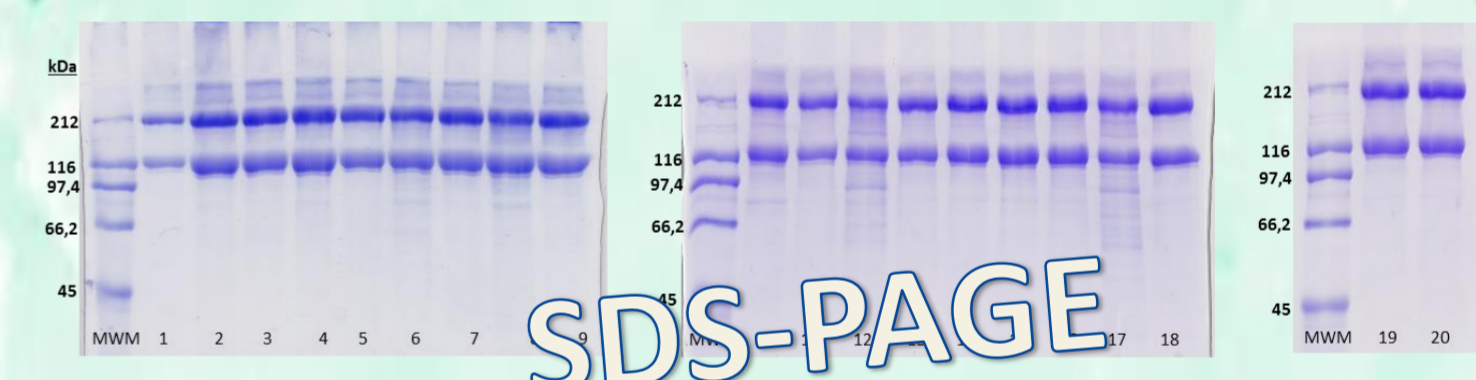


Undigested skin residue washing



Second experimental design: Acetic acid extraction

Experiment number	T (°C)	Acetic acid (M)	t (h)	T cod	Acetic cod	t cod
1	8.26	0.36	11.33	-1.000	-1.000	-1.000
2	20.74	0.36	11.33	1.000	-1.000	-1.000
3	8.26	0.84	11.33	-1.000	1.000	-1.000
4	20.74	0.84	11.33	1.000	1.000	-1.000
5	8.26	0.36	38.67	-1.000	-1.000	1.000
6	20.74	0.36	38.67	-1.000	-1.000	1.000
7	8.26	0.84	38.67	-1.000	1.000	1.000
8	20.74	0.84	38.67	1.000	1.000	1.000
9	4.00	1.00	25.00	-1.682	0.000	0.000
10	25.00	1.00	25.00	1.682	0.000	0.000
11	14.50	0.20	25.00	0.000	-1.682	0.000
12	14.50	1.00	25.00	0.000	1.682	0.000
13	14.50	0.60	2.00	0.000	0.000	-1.682
14	14.50	0.60	48.00	0.000	0.000	1.682
15	14.50	0.60	25.00	0.000	0.000	0.000
16	14.50	0.60	25.00	0.000	0.000	0.000
17	14.50	0.60	25.00	0.000	0.000	0.000
18	14.50	0.60	25.00	0.000	0.000	0.000
19	14.50	0.60	25.00	0.000	0.000	0.000
20	14.50	0.60	25.00	0.000	0.000	0.000



RESULTS AND DISCUSSION

Experimental design for NaOH stage

The combined effect of **NaOH concentration**, **time** and **temperature** (as independent variables) on the amount of **collagen recovered** in the undigested skin residues, together with the minimal loss of collagen in the filtrated solution after the treatment (determined by the nitrogen content in each fraction), was studied by means of **Response Surface Methodology**. The values that maximize the recovery of nitrogen and the removal of proteins different than collagen in the skin residues were **8,3°C**, **11 h** and **0,49 M NaOH**.

Experimental design for acetic acid extraction

The skins treated under the optimal NaOH conditions obtained, were subjected to a second experimental design, to study the combined effect of **acetic acid concentration**, **time** and **temperature** on the **collagen recovery** by means of yield, amino acid content (HyPro and Pro) and SDS-PAGE characterization. In general, no differences were observed in the electrophoretic patterns showing β and γ components in all treatments, however preliminary analysis showed that low temperatures and concentration of acetic acid as well as reduced times of incubation negatively influences the collagen yield.

CONCLUSIONS

This is the **first study** in which **response surface methodology** was performed to investigate the effects of extraction parameters on **Acid Soluble Collagen (ASC)** using the skins of the highly discarded *Scyliorhinus canicula* shark. The results obtained would be helpful for scaling up the process to an industrial scale accomplishing one of the main purposes of UE fishing policies pursuing the **full use of fishing captures**.

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