Collagen extracted from Asian sea bass and diatoms purified silica for the development of scaffold for bone regeneration

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INTRODUCTION

The use of marine and freshwater resources to obtain valuable compounds is an approach receiving more attention in recent years under the scope of sustainable exploitation of these resources – namely under the concept of Circular Economy - adding value to the current management of fish by-products and microalgae that is mainly associated with animal feed.

Collagen represents the main structural protein of the various connective tissues in animals, accounting for approximately 30% of all vertebrate body protein, and can be extracted or isolated from different sources[1]. Fish collagens are increasingly reported in the literature and being considered highly attractive by the industry as an important alternative source of collagen for different fields of application including food, cosmetics, pharmaceutical and biomedical[2].

The silica from diatoms have a combination of structural, mechanical and chemical characteristics that make them a promising source in the development of new and naturally based solutions for the biomedical area[3-5] .

This work looked particularly into Asian sea bass scales for the extraction of collagen, using acetic acid (ACoII) and pepsin (PColl) based methodologies, as well as to diatoms produced in aquaculture, which were used to recover silica by thermal purification.

MATERIALS AND METHODS

Acid and Pepsin extraction of collagen from Asian sea bass scales

Asian seabass scales → Degumming with a 10% NaCl solution

↓ Remove non-collagen proteins with NaOH

↓ Decalcification using 0.1M HCl

↓ Acid extraction (ACoII)

↓ Re-extraxtion with 0.5% pepsin (PColl) → Precipitacion with NaCL → Dialyse → Freeze drying

Figure 1. Asian sea bass scales.

Thermal purification of silica from diatoms

Figure 2. Diatoms after centrifugation.

Diatom

↓ Centrifugation (3000 rpm, 20 min, RT).

↓ Thermal treatment (600ºC)

↓ Rinsed with distilled water (5x)

↓ Dried at 60ºC

Characterization

The resulting material will be characterized regarding structural, (bio)chemical and morphological information properties:

Collagen

- ✓ SDS-PAGE;
- ✓ FTIR-ATR;
- ✓ CD;
- ✓ Viscosity;
- ✓ ….

Silica

- ✓ TGA;
- ✓ FTIR-ATR;
- ✓ SEM/EDS;
- ✓ ….

RESULTS AND DISCUSSION

Collagen from Asian sea bass scales

✓ **The extraction of collagen was possible from Asian sea bass scales through the referred methods having an extraction yield of 1% and 6.6% for ACoII and PColl respectively.**

✓ **SDS-PAGE** profile is consistent with type I collagen

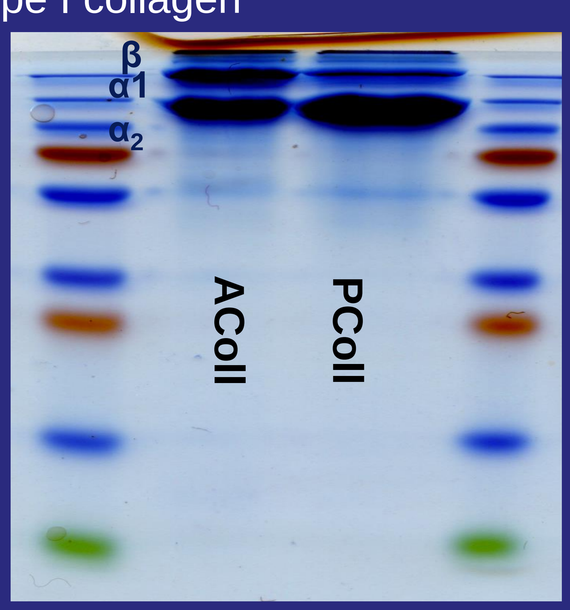


Figure 3. Electrophoresis analysis of collagen extracts.

✓ **FTIR** spectrum of ACoII and PColl exhibited the characteristic peaks of collagen.

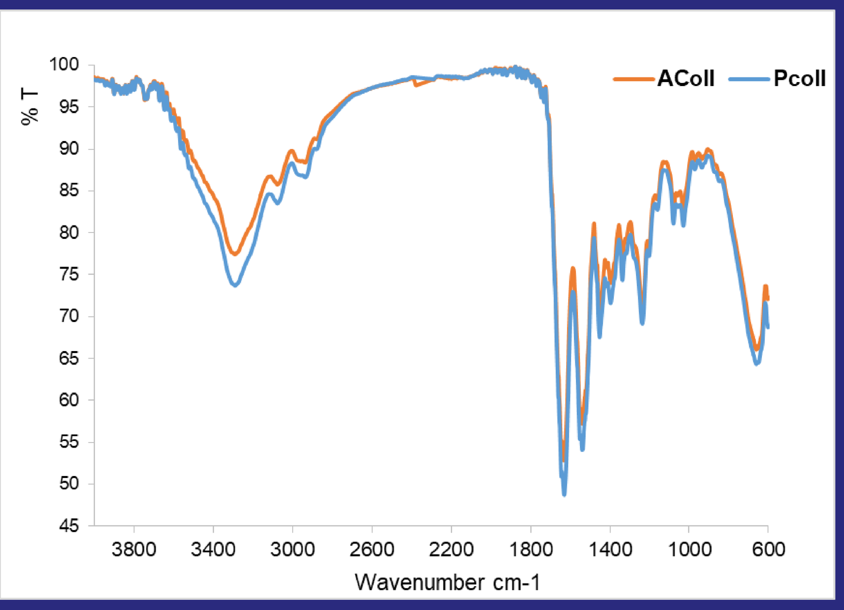


Figure 4. FTIR-ATR spectra of ACoII and PColl.

✓ **CD suggest that Acoll and PColl structure remained intact, with preserved triple helix.**

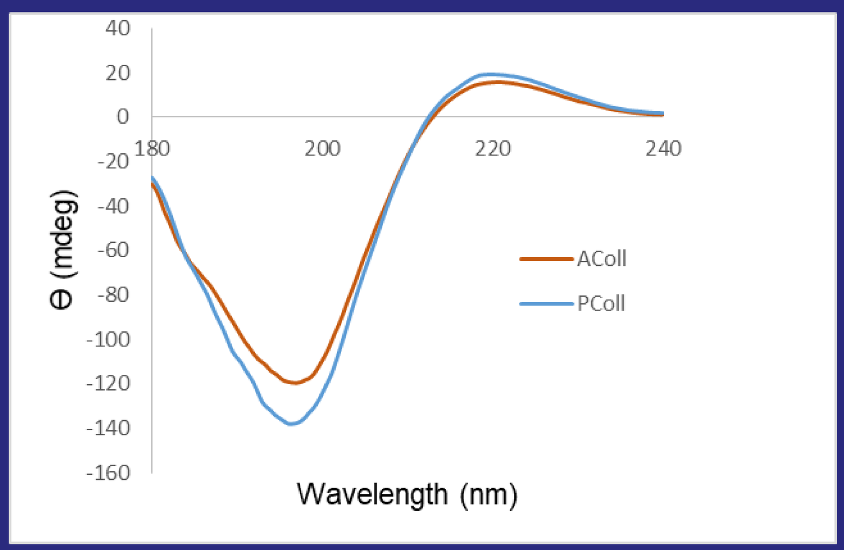


Figure 5. CD spectra of ACoII and PColl measured at 26 ºC.

✓ **Statistical analyses from CD results confirmed that the denaturation temperature was 44,56ºC with R^2=0,998 in ACoII sample and was 44,89ºC with R^2=0,998 in PColl sample.**

✓ **The viscosity of the collagen samples (0,5%) were measured through a Digital Viscometer which showed that the extracts are non-Newtonian fluids, having a viscosity of about 3 mPa/s for both ACoII and PColl.**

Silica from diatoms

✓ **Thermal gravimetric analysis (TGA) was performed to address thermal stability of diatoms**

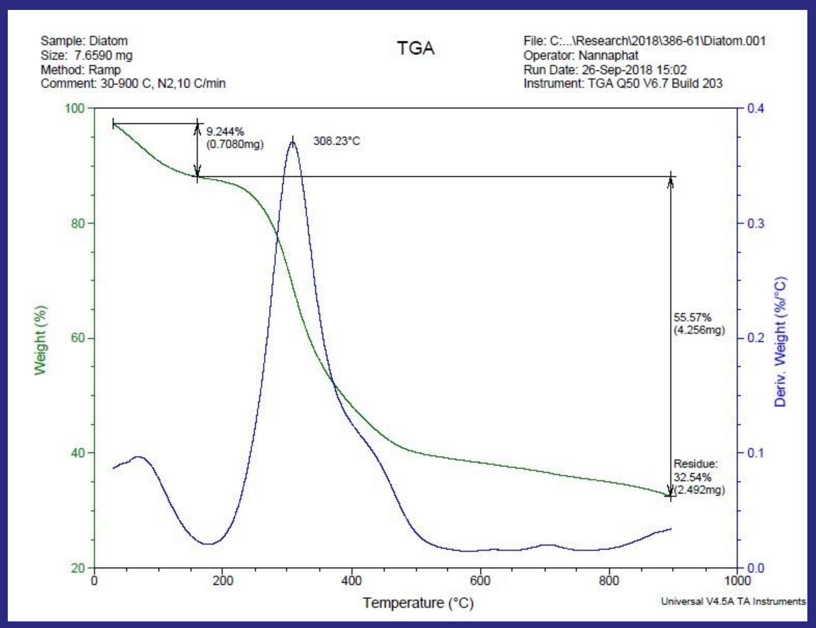


Figure 6. Thermogravimetric weight loss plot and derivative weight loss curve of the original diatom cells.

✓ **There are 2 main weight loss stages:**

1. **Evaporation of body water and physically absorbed water.**
2. **Degradation of the organic compounds.**

✓ **FTIR-ATR and SEM/EDS** analysis confirming the presence of Si in the SiO2 form, and that the structure of the frustules has been destroyed.

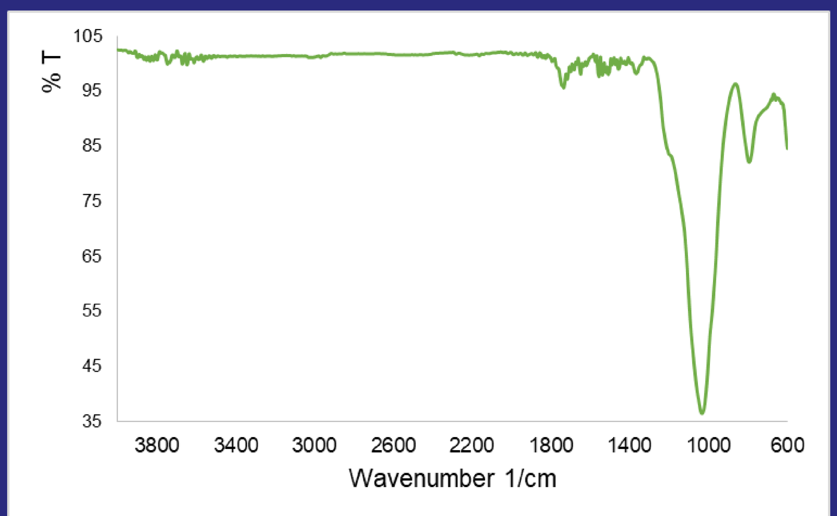
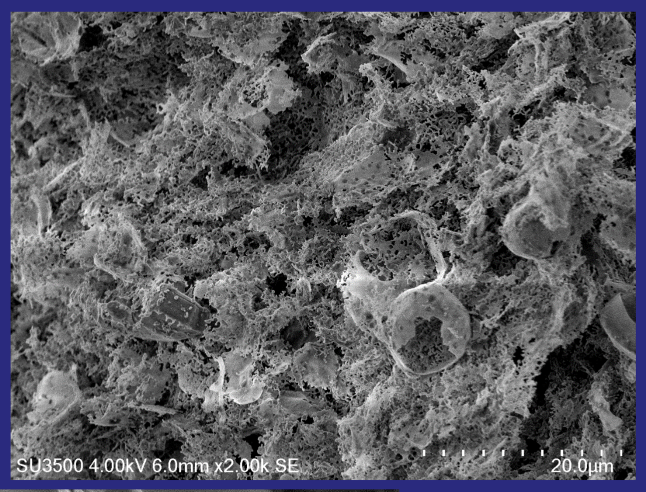
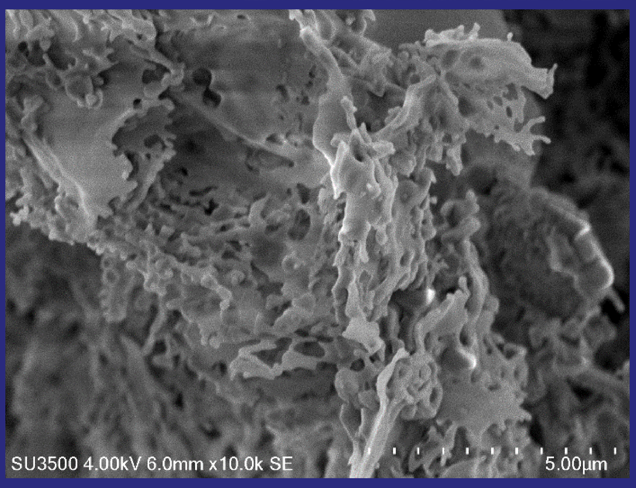
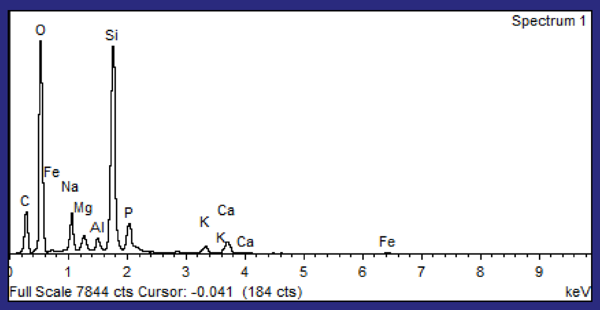


Figure 7. FTIR-ATR spectra of silica from diatom







Element	Weight%	Atomic%
C	31,49	41,24
O	40,80	42,08
Na	2,44	1,79
Mg	0,87	0,61
Al	0,79	0,51
Si	17,07	10,69
P	2,43	1,36
K	1,34	0,61
Ca	1,93	0,85
Fe	0,85	0,27
Totals	100,00	

Figure 8. SEM/EDS analyses of silica from diatoms.

CONCLUSIONS

The produced materials have shown interesting results looking into their future use as building blocks for the production of biomaterials for biomedical applications. In particular, their use in scaffolding for bone regeneration is currently being addressed.

❖ ACoII and PColl were successfully extracted from Asian sea bass scales and classified as type I collagens, according with the SDS-PAGE profile; The results of the FTIR-ATR and CD analyses indicate that extracts maintained its native triple helix structure; Extraction with pepsin increase the collagen production yield; The collagen extracts obtained present interesting rheological properties envisaging their biomedical application, namely to be processed further as injectable biomaterials.

❖ Through the EDS and FTIR-ATR spectrum it is possible to confirm the presence of Silica in the SiO_2 form, being one of the principal constituents of the sample; Optimization of the purification process is on-going in order to remove impurities and minimize damage to the silica structure.

Further characterization methods need to be performed to better understand diatom silica functionality after thermal purification and collagen functionality and morphology.

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