

Collagen extraction from Asian sea bass and silica purification from diatoms envisaging the development of scaffolds 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 (AColl) 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

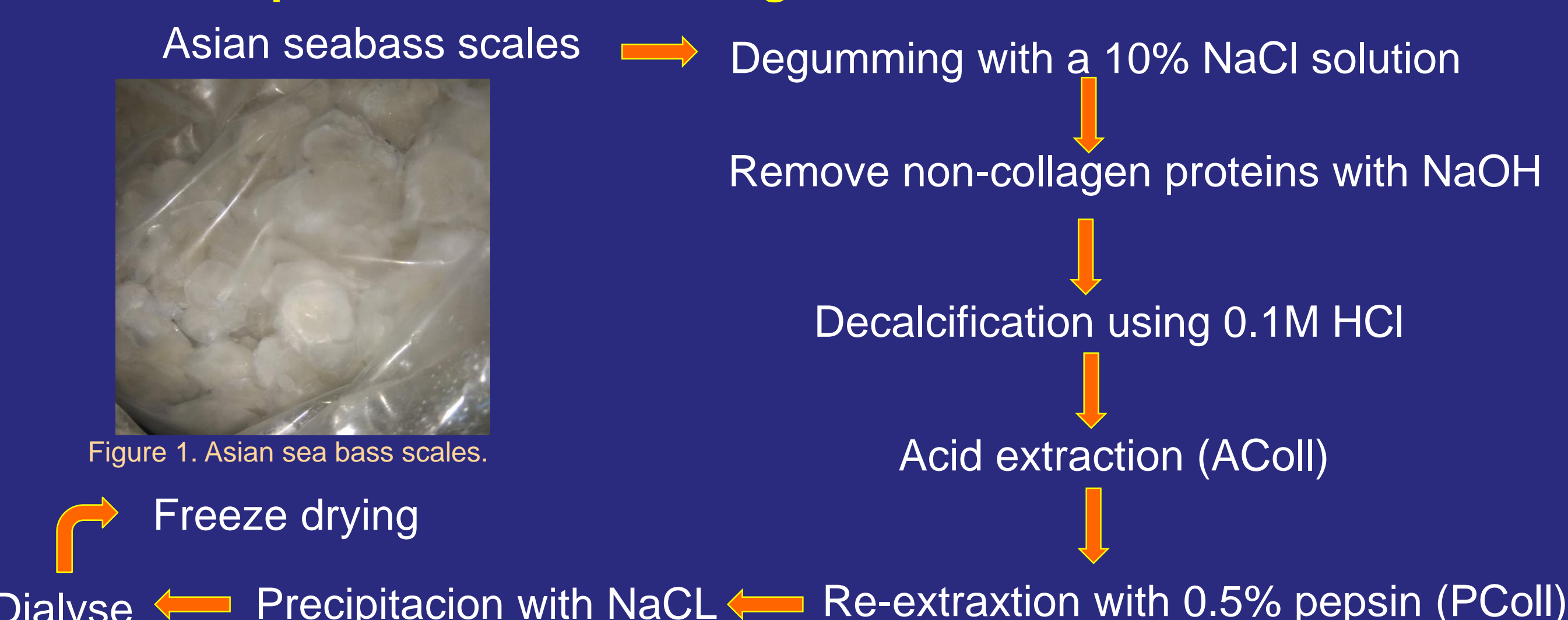
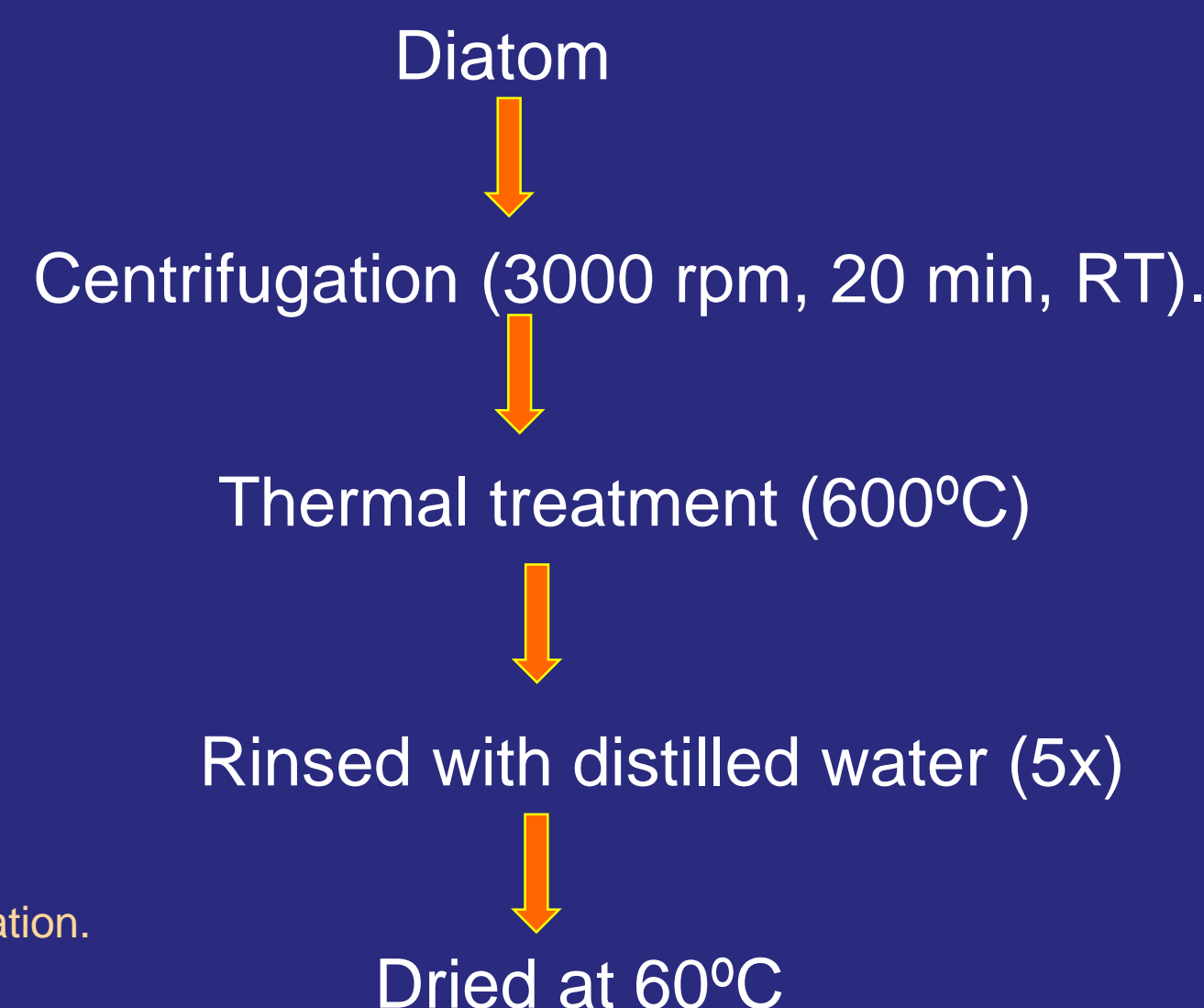


Figure 1. Asian sea bass scales.

Thermal purification of silica from diatoms



Figure 2. Diatoms after centrifugation.



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 AColl and PColl respectively.

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

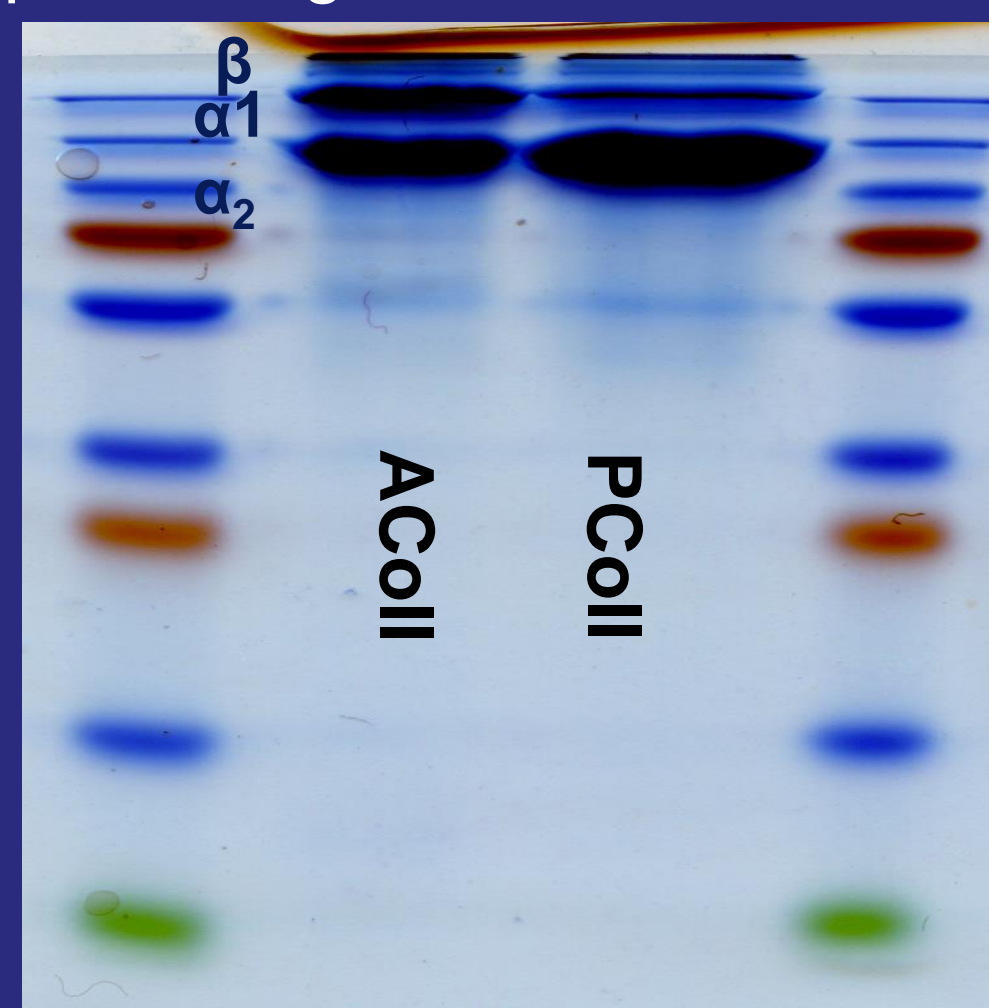


Figure 3. Electrophoresis analysis of collagen extracts.

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

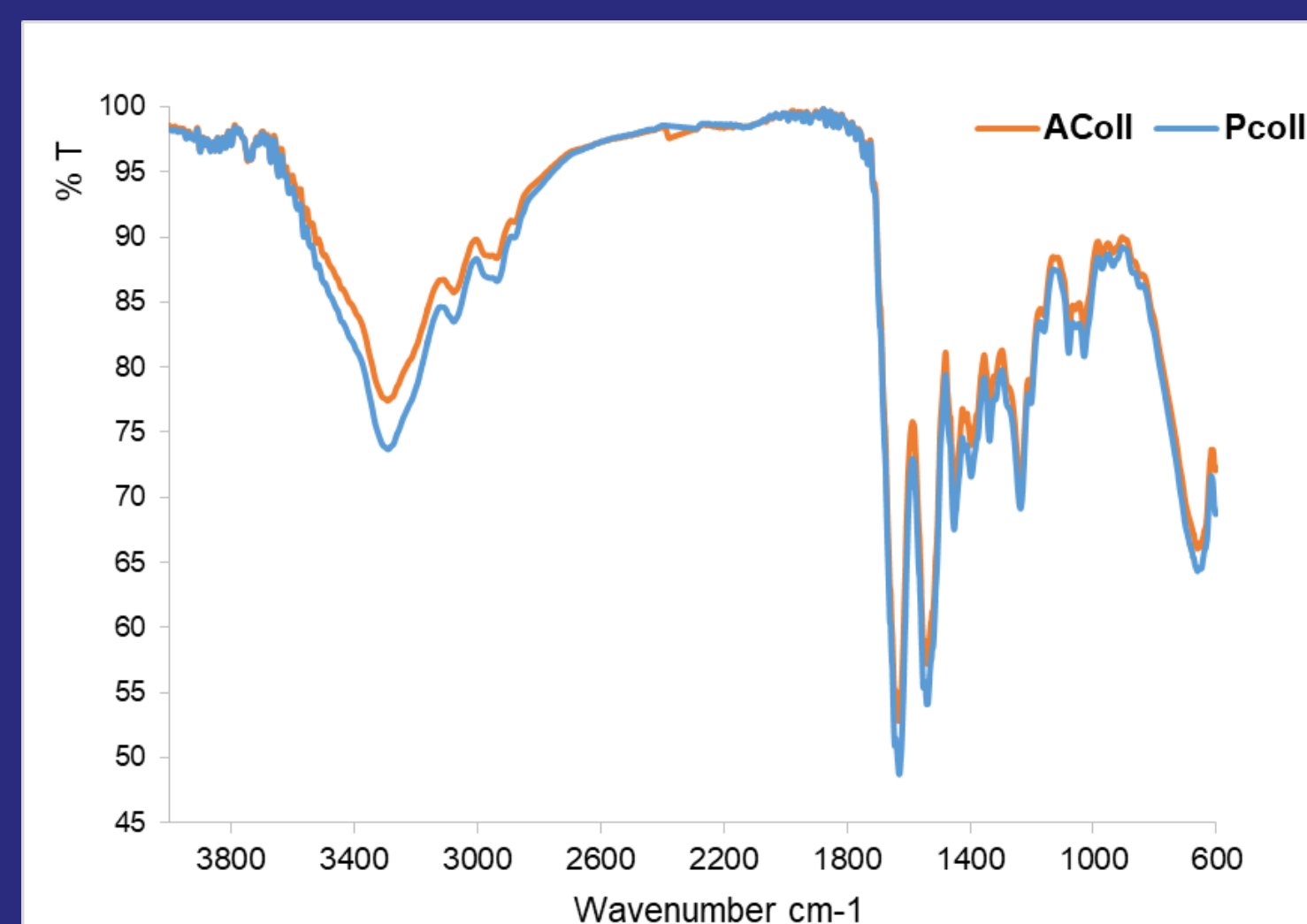


Figure 4. FTIR-ATR spectra of AColl and PColl.

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

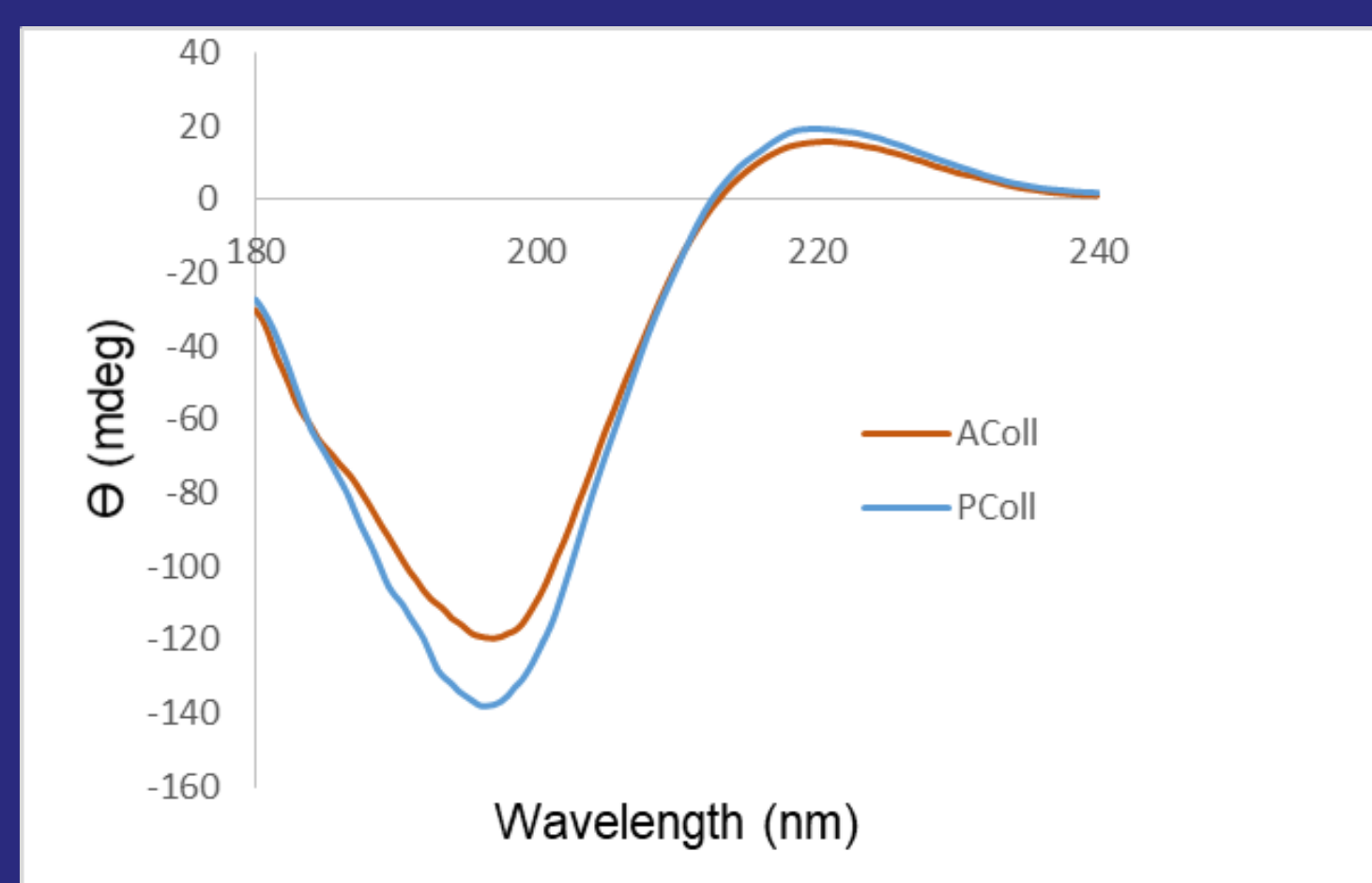


Figure 5. CD spectra of AColl and PColl measured at 26 °C.

✓ Statistical analyses from CD results confirmed that the **denaturation temperature** was 44,56°C with R²=0,998 in AColl sample and was 44,89°C with R²=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 AColl and PColl.

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

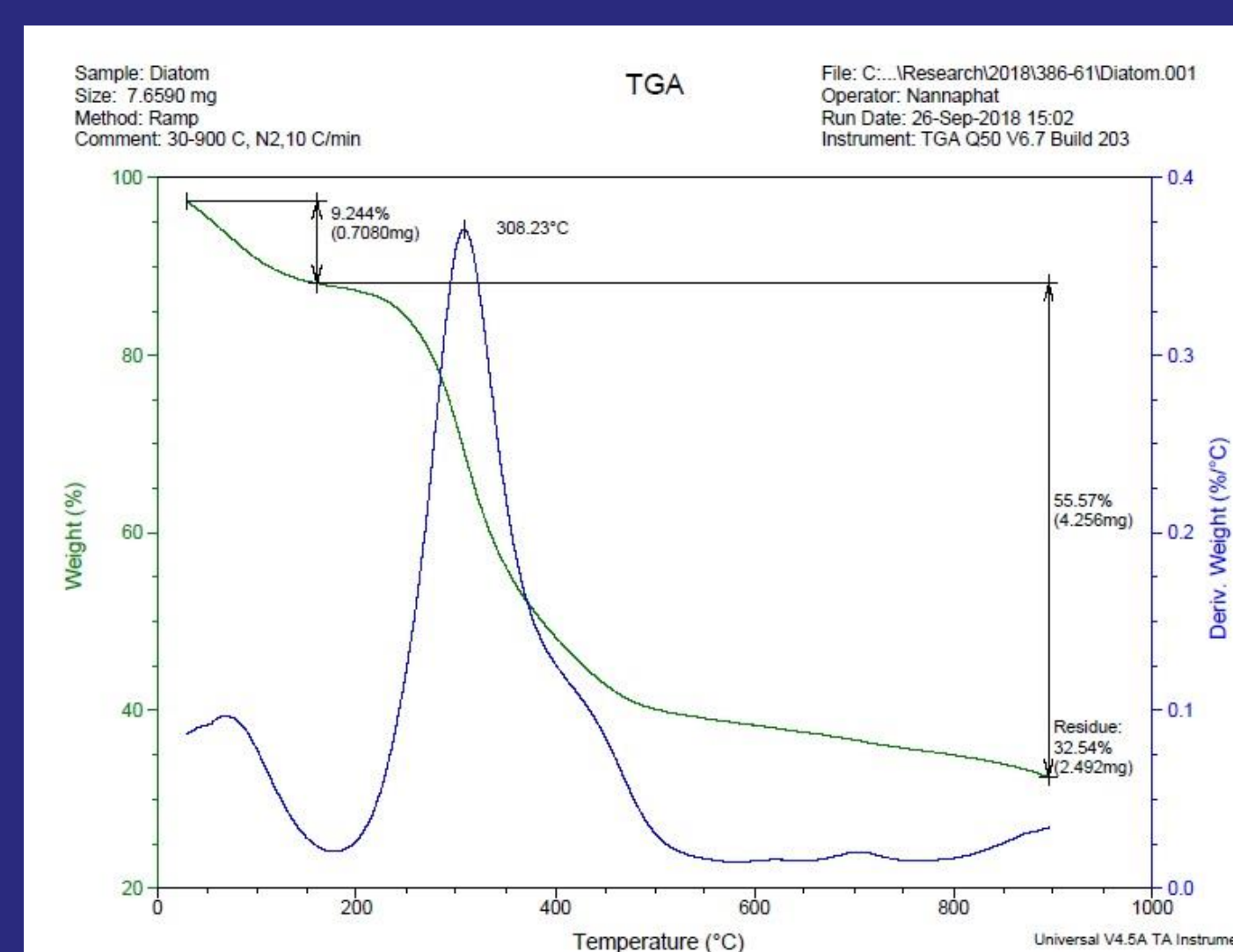
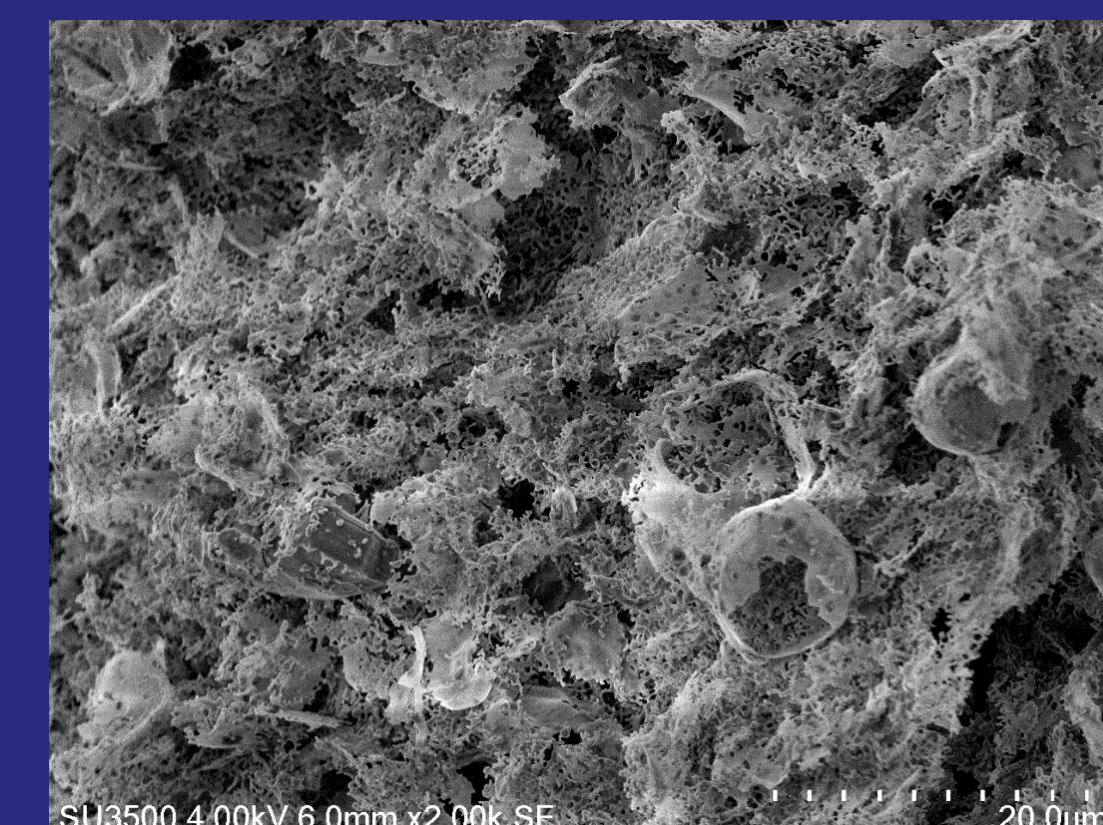


Figure 6. Thermogravimetric weight loss plot and derivative weight loss curve of the original diatom cells.

✓ 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.

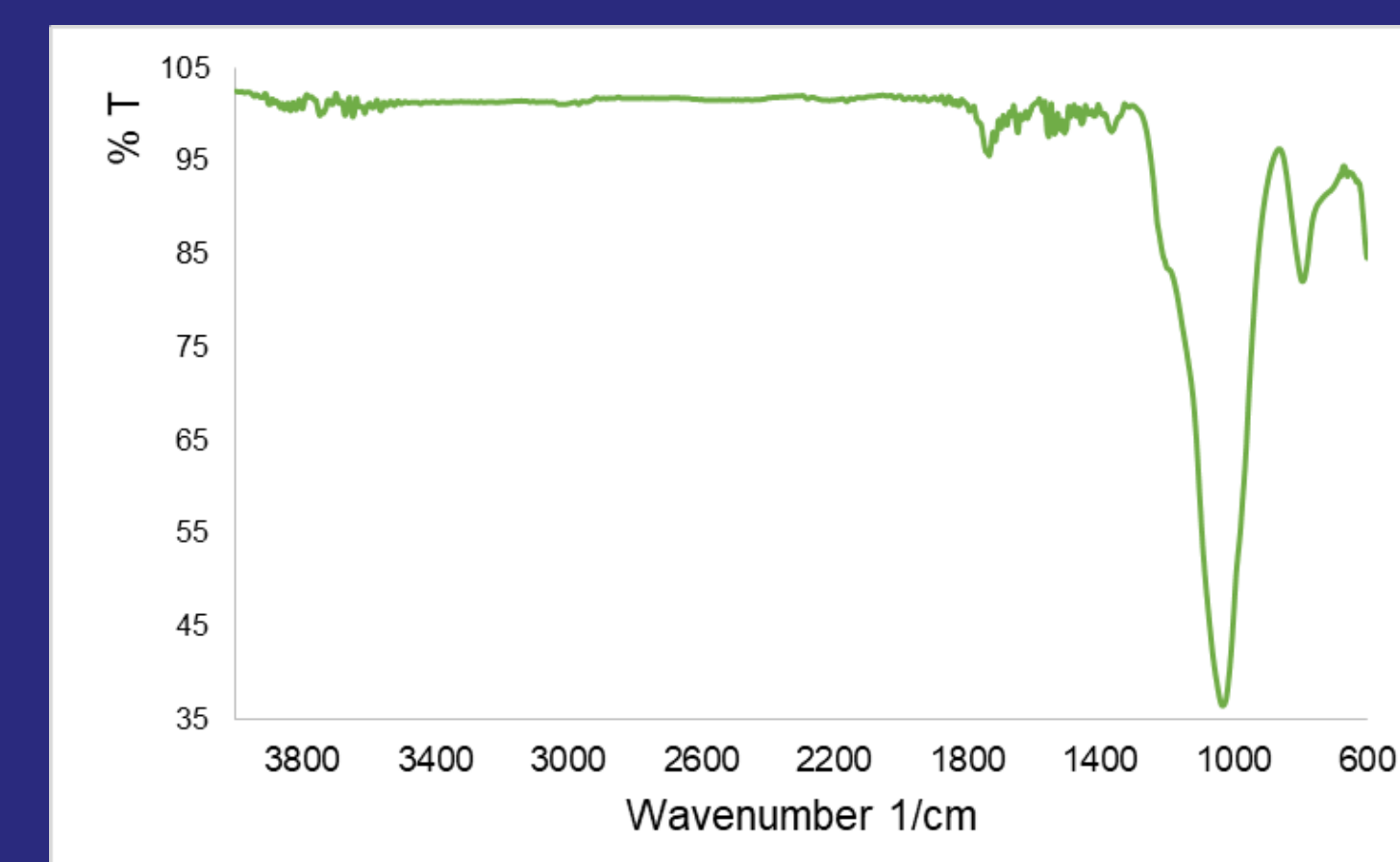
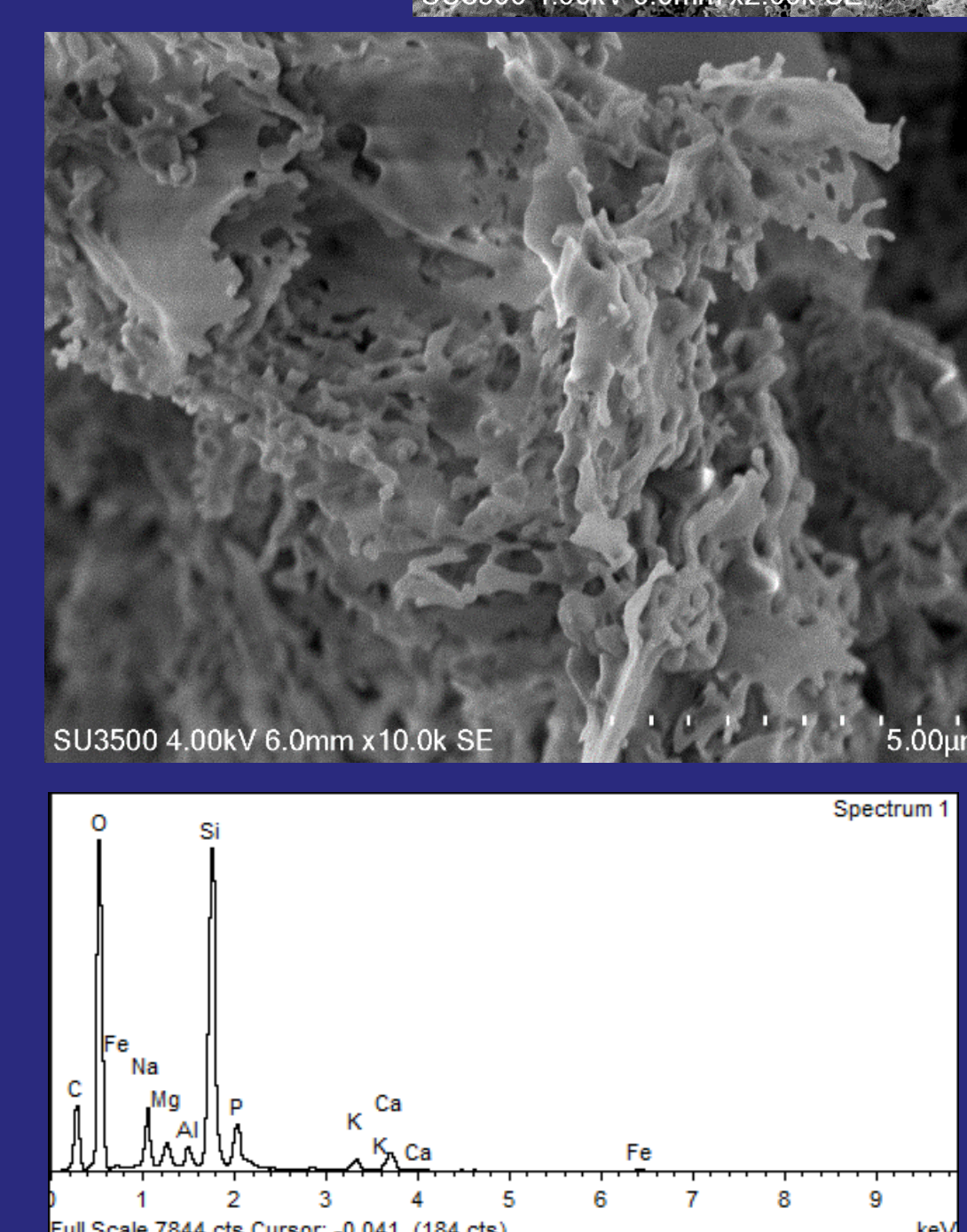


Figure 7. FTIR-ATR spectra of silica from diatom



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.

❖ **AColl** 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 SiO₂ 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.

References:

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Acknowledgments: Financial support from European Regional Development Fund (ERDF), through European Union Transborder Cooperation Programme Interreg España-Portugal 2014-2020 (POCTEP), under the scope of project 0302_CVMAR_I_1_P and from European Union's Horizon 2020 under the MSCA-RISE instrument, Grant agreement 778078 – REMIX Project..