ANCOVA 17

Aquaculture Nouvelle de COncombre de mer, de Ver tube et d'Algue rouge en Charente-Maritime

Exploration des molécules d'intérêt du ver tube *Diopatra biscayensis* et des concombres de mer *Holothuria sp*

Rapport de synthèse

Rédaction : Pierrick BARBIER
Collaboration : Cédric HENNACHE, Laura VASSEUR

Février 2025

Pierrick BARBIER, Cédric HENNACHE, Laura VASSEUR

Centre pour l'Aquaculture, la Pêche et l'Environnement de Nouvelle-Aquitaine

Exploration des molécules d'intérêt du ver tube *Diopatra biscayensis* et des concombres de mer *Holothuria sp*

Rapport de synthèse
37 pages

Février 2025

Barbier P., Hennache C., Vasseur L. (2025) Exploration des molécules d'intérêt du ver tube *Diopatra biscayensis* et des concombres de mer *Holothuria sp.* Projet ANCOVA 17, Rapport de synthèse, CAPENA. 37p

RÉSUMÉ :

Le projet ANCOVA17 a pour objectif d'étudier les opportunités de développement de nouvelles filières d'aquacultures marines en Charente-Maritime.

L'axe de travail 4 concerne particulièrement la recherche de molécules d'intérêt en vue d'une exploitation commerciale au niveau national et international. Ce travail est réalisé en partie par l'équipe BCBS Biotechnologie et Chimie des Bioressources pour la Santé de La Rochelle Université. Par l'intermédiaire de plusieurs stages, ce laboratoire a la charge d'identifier des molécules d'intérêt potentiellement présentes dans le vers tube et les concombres de mer pour les secteurs de la bio-pharmaceutiques, de la cosmétique, ou encore le secteur médical.

Une synthèse des résultats clés est présentée dans ce document, auquel les rapports de stage sont annexés.

Aquaculture ; Charente-Maritime ; Diversification ; Nouvelle filière ; Production ; Molécules d'intérêt ; Extraction

Table des matières

I. Introduction

Dans le cadre du projet ANCOVA 17, des études approfondies ont été menées sur deux organismes marins prometteurs : le ver tube *Diopatra biscayensis* et les concombres de mer du genre *Holothuria*. L'objectif était d'identifier et d'exploiter les molécules d'intérêt présentes dans ces organismes pour des applications potentielles en valorisation biologique, médicale et industrielle.

Ces études reposent en partie sur une **analyse proximale**, une méthode qui permet d'évaluer la composition globale d'un organisme ou d'un produit biologique. Cette analyse comprend la mesure de la matière sèche, des cendres (minéraux), des protéines, des lipides et des glucides. Elle fournit des informations essentielles pour comprendre le potentiel nutritif et fonctionnel des organismes étudiés et orienter leur valorisation.

II. Analyse des molécules d'intérêt chez les concombres de mer (*Holothuria sp.*)

Les travaux réalisés à l'Université de La Rochelle se sont concentrés sur la caractérisation proximale des concombres de mer *Holothuria forskali* et *Holothuria tubulosa*, avec un accent particulier sur l'extraction du collagène (Annexe 1).

1. Autres composés bioactifs

Les concombres de mer sont riches en polysaccharides sulfates (*fucosylated chondroitin sulfate* - FCS), saponines et composés phénoliques.

- **FCS et fucoïdanes** : propriétés antioxydantes, anticoagulantes et anti-inflammatoires.
- **Saponines (frondoside A)** : activités immunomodulatrices, anticancéreuses et neuroprotectrices.
- **Composés phénoliques** : rôle potentiel dans la lutte contre le stress oxydatif et l'inflammation.

2. Composition biochimique

Les analyses ont révélé que la peau des concombres de mer est principalement constituée de protéines (~50 % du poids sec), de minéraux et d'un faible taux de lipides et de sucres (<1 %). Les quantités extraites des différentes molécules bioactives sont les suivantes :

- **Collagène** : Extraction maximale obtenue avec l'acide acétique, représentant environ **30 % des protéines totales**.
- **Sucres** : Les analyses par la méthode de Dubois indiquent que les concombres de mer contiennent environ **0,25 % de sucres réducteurs solubles**.
- **Lipides** : Présence négligeable (<1 %), confirmant que les concombres de mer ne sont pas une source lipidique exploitable.

3. Extraction et valorisation du collagène

L'extraction du collagène a suivi plusieurs étapes méthodologiques précises :

1. **Prétraitement** : Délipidation des échantillons par bain d'éthanol à 90 % pendant 2 heures.
2. **Décalcification** : Utilisation d'une solution d'acide acétique 0,5M pour solubiliser le collagène tout en préservant sa structure.
3. **Filtration et concentration** : Séparation des fractions solubles et précipitation du collagène par modification du pH.
4. **Caractérisation** : Électrophorèse pour évaluer l'intégrité et la pureté du collagène extrait.

Les résultats indiquent que :

- L'acide chlorhydrique était trop agressif, entraînant une dégradation des protéines.

- L'acide lactique montrait un rendement insuffisant.
- L'acide acétique offrait un bon compromis et a permis l'extraction d'un collagène exploitable avec une structure bien préservée.

4. Perspectives de valorisation

Les concombres de mer représentent une ressource intéressante pour l'industrie pharmaceutique, cosmétique et nutraceutique. De plus, leur capacité à purifier leur environnement par bioremédiation en fait un candidat idéal pour l'Aquaculture Multi-Trophique Intégrée (AMTI).

III. Analyse des molécules d'intérêt chez le ver tube (*Diopatra biscayensis*)

L'étude menée sur les vers tubes visait à analyser la composition chimique de leurs structures (vers et tubes) et à explorer leur potentiel de valorisation (Annexe 2).

1. Composition biochimique

Trois échantillons ont été étudiés :

- **Tubes sales** (avec coquilles et fibres)
- **Tubes propres** (structure lavée)
- **Vers eux-mêmes**

Les résultats de l'analyse proximale montrent :

- **Taux de matière sèche** : ~28-29 % pour les tubes, ~23 % pour les vers.
- **Teneur en cendres** : très élevée dans les tubes sales (~80 %) en raison de la présence de coquilles, et plus faible dans les vers (~30 %).
- **Teneur en protéines** : ~15 % pour les tubes propres et ~14 % pour les vers (sur poids sec).

2. Extraction et solubilisation des protéines

Les tests de solubilisation montrent que :

- Les protéines des vers sont relativement bien solubles.
- Les tubes sont insolubles dans l'eau et l'éthanol mais peuvent être solubilisés en acide chlorhydrique 6M.
- L'électrophorèse a révélé que seules les protéines des vers sont en quantité suffisante pour être visualisées.

3. Contenu en glucides

Les analyses par la méthode de Dubois ont montré que les tubes contiennent environ **26 % de polysaccharides** sur poids sec, tandis que les vers en contiennent très peu (~1,4 %).

IV. Conclusion générale

Les études menées dans le cadre du projet ANCOVA 17 démontrent le fort potentiel des concombres de mer et des vers tubes pour une valorisation biologique et industrielle.
Ces travaux ouvrent la voie à des applications innovantes dans les domaines de la nutraceutique, de la biotechnologie et de l'économie circulaire appliquée aux ressources marines.

V. Annexe 1

Nur MUHTAROĞLU (2024) Sea Cucumber Proximal Characterization and Collagen Extraction. Rapport de stage, BCBS team, LIENSs, La Rochelle Université. Supervision Stéphanie Bordenave-Juchereau

Sea Cucumber Proximal Characterization and Collagen Extraction

La Rochelle University
23 Av. Albert Einstein, 17000 La Rochelle

BCBS Team
Biotechnologie et Chimie des Bioressources pour la Santé

Nur MUHTAROĞLU

External Supervisor: **Stéphanie Bordenave-Juchereau**
stephanie.bordenave@univ-lr.fr

I. ABSTRACT

This report includes research and practical experimentation on characterising the sea cucumbers content and tentative collagen extraction. The experiments are conducted the laboratory in La Rochelle University in La Rochelle, France.
The characteristics of sea cucumbers are analysed measuring dry weight, ash, lipid, protein and reducing sugar content.
The fresh skin of sea cucumber contained 10% of dry matter whereas when it was frozen, the value was 6%. Almost no lipids neither sugars were detected (less than 1% w/w). As expected, the main part of the skin contained proteins (around 50%) and minerals.
The collagen extraction process was first carried out with HCl, lactic acid and acetic acid at cold temperature. HCl was too strong whereas lactic acid was too weak to extract proteins. Then, to optimise collagen extraction, two methods were tested. The first group has been used to conduct a wet extraction without drying sea cucumbers first and the second group has been tested on dried sea cucumbers skins. Wet sea cucumbers in average have a dry weight content of 5,53%, lipid content of 1,03% and 16% of protein. While for the dried sea cucumbers these values were 83,66% of dry weight, 2,03% of lipid and 34% of protein.
Dry extraction process was more efficient and yielded in higher protein concentration which seems logical.
Some collagen was extracted and characterised by electrophoresis, but with low yields and degraded quality (smelly brown powder). This may be due to the degradation of the collagen during the storage process and a lack of pigment removal.

These results showed that sea cucumbers *H. forskali* and *H tubulosa* are not suitable for extracting neither lipids and polysaccharides but only for protein extraction like collagen even this technic should be adapted to this pigmented resource.

II. INTRODUCTION

1. Overview of sea cucumber cultivation

Sea cucumbers are benthic marine invertebrates'organisms and belong to the class of Holothuroidea and the phylum of Echinodermata (Hossain et al. 2020). They are widely consumed in China, Korea, Japan, Malaysia, Indonesia, and Russia (Hossain et al. 2020).
In the world, the most common commercial species are *Apostichopus japonicus*, *Acaudina molpadioides, Actinopyga mauritiana, Cucumaria frondosa*, *Holothuria polii, Holothuria nobilis, Holothuria tubulosa*, *Isostichopus badionotus,* and *Pearsonothuria graeffei* (Hossain et al. 2020). The most common sea cucumbers found in the North Pacific and Atlantic areas are *Cucumaria frondosa*, *Parastichopus californicus, Holothuria forskali*, and *Parastichopus parvimensis*. The species of interest for our study are *Holothuria forskali* and *Holothuria tubulosa.*

The sea cucumber *Cucumaria frondosa*, which is also called « orange-footed sea cucumber » or the « Northern sea cucumber » is the most abundant and widely distributed species in the cold waters of the North Atlantic Ocean and was thus well studied (Hossain et al. 2020).
His geographical distribution is very large, such as in Groenland, Iceland, Canada, Norway. Sea cucumbers are part of the eating habits of Northern countries populations. This resource has been fished for subsistence for centuries and is essential in the life of indigenous people of Canada. Since 1988, fishery of *C.frondosa* is exerced in the Gulf of Maine (North America) and more later, this fishery was extended to Atlantic Canada (Nelson et al. 2012).
In 1994, Asian markets began to accept this product. Actually, the fishery of sea cucumber represents a high value of a low-financial value product and precautionary measures have been put in place to manage *C.frondosa* fisheries.
In parallel to aquaculture, certain sea cucumbers are commonly cultivated through aquaculture systems and co-culture operations. *C.frondosa* is not yet much cultured (Nelson et al. 2012) and this will be soon the case of *Holothuria forskali,* and *Holothuria tubulosa.*

Sea cucumbers are used as healthy foods due to their high content in nutrients (amino-acids). They are also used in traditional medicines to fight asthma, hypertension, arthritis, constipation, anemia and dietary supplements. Numbers of studies have shown that co-products of this sea cucumber have medicinal potential due to the panel of biological activities such as antioxidant, anticancer, anticoagulant, antithrombotic, and antimicrobial effects (Hossain et al, 2020).

2. Composition of sea cucumber

Sea cucumbers are composed of diverse elements such as macronutrients (proteins, lipids, polysaccharides), micronutrients (minerals), bioactives compounds.
The regions of their body structure which are mainly consumed are soft-bodied, cucumber-like, with leathery skin, elongated, and worm-like body (body wall is the main part, which contains around 85% moisture).
His regenerative capacities have been associated with high levels of phospholipids, particularly in free fatty acids and EPA. However, it contains very low fat and cholesterol. Studies have shown that EPA (Eicosapentaenoic acid) is the most abundant fatty acid, comprising 35 % of the total fatty acid in muscle.
In terms of amino acids, glutamic acid is the most abundant, followed by glycine, aspartic and alanine acid. Essentials amino acids are also present in high levels (Ramalho et al. 2020). It possesses a high protein content. In fact, after saponins, proteins are the second predominant element in this cucumber.

C. frondosa contains relatively high levels of calcium, selenium, and zinc, and very high levels of iron, potassium, sodium, and phosphorus (Ramalho et al. 2020).

	Parameter	Concentration
Macronutrients	Total protein (g/100 g DW [1])	43.9 ± 0.2
	Glycogen (g/100 g DW [1])	6.2 ± 0.8
	Total lipids (g/100 g DW [1])	32.2 ± 0.4
	Cholesterol (µg/g DW [1])	0.43 ± 0.04
Minerals (µg/g DW [1])	Calcium	1350 ± 71
	Iron	88 ± 3
	Potassium	20,000 ± 0
	Sodium	19,000 ± 0
	Phosphorus	11,000 ± 0
	Selenium	2.25 ± 0.07
	Zinc	63.5 ± 3.5
Bioactive compounds	Carotenoids (µg/g DW [1])	636.1 ± 24.3
	Saponins (mg/g DW [1])	178 ± 17
Arsenic (µg/g DW [1])	Organic	5.90
	Inorganic	1.69
Other	Humidity (g/100 g DW [1])	7.68 ± 0.10
	Peroxide value (PZ) (mEq/kg humid product)	4.39 ± 0.64
	P-anisidine value (p-AnV)	30.3 ± 3.5
	Total oxidative value (2 PV + p-AnV)	39.1 ± 2.3

[1] DW = dry weight.

Table 1: Composition of *Cucumaria frondosa* (from Ramalho et al. 2020)

This sea cucumber also contains bioactive components, comprising polysaccharides that can exhibit complex structure and various functional activities, but also proteins and pigments. The dermis of *Cucumaria frondosa* contains collagen fibrils.
Previous tests performed revealed that, although the concentrations of lead, cadmium, and mercury were well below acceptable limits, the concentrations of total arsenic and especially inorganic arsenic were close to the maximal acceptable limits.

This capacity to accumulate heavy metal could be one the of the bottleneck to the consumption of this animal (Ramalho et al. 2020).

Let's analyse the proximal composition of sea cucumbers.

2.1. Polysaccharides

The body wall of sea cucumber is supposed to be very rich in acidic polysaccharides, mainly in sulfated polysaccharides. Two types of polysaccharides were identified: Fucosylated Chondroitin Sulfate (FCS) and fucans (Hossain et al. 2020) (Figure 2).

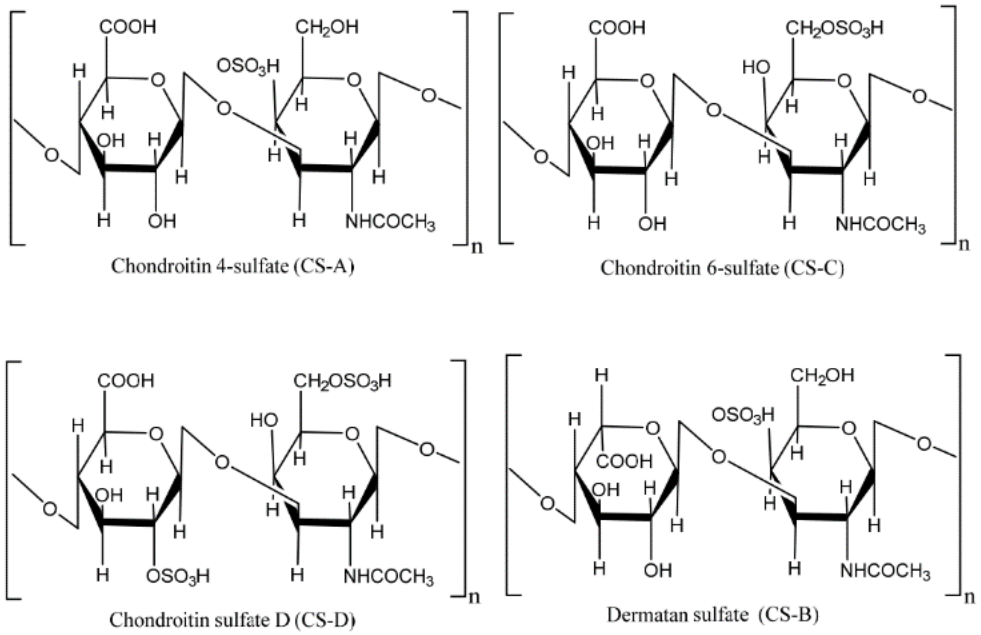


Figure 2 : Structure of Fucosylated Chondroitin Sulfate (FCS) and principal forms in nature (Hossain et al. 2020)

FCS is a unique glycosaminoglycan found in sea cucumber. Studies show that its bioactivity depends on the degree of sulfation of monosaccharide composition, and the position of the sulfated group. Chondroitin Sulfates (CS) are composed of repeating disaccharide units : (a) glucuronic acid and (b) N-acetylated galactosamine (Hossain et al. 2020).
FCS is associated with the anticoagulant as well as antithrombotic activities. It has been reported that anticoagulant properties generally depend on the sulfate content of FCS (Hossain et al. 2020).
FCS from sea cucumbers may have plenty of biological activities, such as anti-inflammatory, anticoagulant, antitumor and and others.
Studies show that the level of biological effect strongly depends on the structural features of FCS. Moreover, isolated from C.frondosa bodywall, FCS have shown an anti-hyperglycemic activity as well as fucoidans (Hossain et al. 2020).

b) Fucoidans:
Fucoidan is one of the most important bioactive compounds in this organism, particularly in the body wall. It comprises L-fucose and sulfate groups (Hossain et al. 2020).
It posseses antithrombotic and anticoagulant properties, and it is able to inhibit osteoclastogenesis, and enhance protection from gastric damage. Furthermore, fucoidans significantly prevented high-fat high-sucrose diet injured pancreatic islets, decreased insulin, tumor necrosis factor (TNF)−α (Janakiram et al. 2015), blood glucose levels, and enhanced adiponectin level (Hossain et al. 2020).
A study realized on insulin resistant mice reveals that fucoidan from *C. frondosa* showed significant anti-hyperglycemic activities via activating phosphatidylinositol 3 kinase (PI3K)/protein kinase B (PKB) pathway and glucose transporter 4 (Wang et al. 2016).
In vitro and *in vivo* studies on renal interstitial fibrosis demonstrated that fucoidans regulate the phosphatidylinositol-3-hydroxykinase/protein kinase-B/nuclear factor-κB (PI3K/AKT/NF-κB) signaling pathway (Song et al. 2022). So, it ameliorates renal function, inhibits inflammation, inhibits fibrosis, and reduces the accumulation of the extracellular matrix by an inhibition of the expression of the protein PI3K (Song et al. 2022).

2.2. Saponins

Saponins are secondary metabolites, composed of triterpene glycosides, so oligosaccharides play a crucial role in chemical defense. These molecules are also named as holostane and

nonholostane and they are formed of a carbohydrate chain composed of up to six monosaccharides. They are commonly known as holothurins, and the major saponin in *C. frondosa* is frondoside A (Hossain et al. 2020).
Frondoside A is certainly the most important molecule produced by *C.frondosa* in terms of biological activity spectrum. It exhibited various biological properties, including antiproliferative, immunomodulatory effects, antiviral, antiprotozoal, antifungal, anticancer, antineoplastic, and antitumor activities (Hossain et al. 2020).
One of the most important examples of bioactivity of saponins extracted from *Cucumaria frondosa* is the limitation of neurodegenerative diseases, such as Alzheimer's disease. In one of these studies, saponins was used to treat transgenic Caenorhabditis elegans model of Alzheimer's disease and investigated its effects on Amyloid-β (Aβ) aggregation and proteotoxicity. Results reveal that the presence of saponins permits a decrease of Aβ aggregation (Liang et al. 2022).
In addition, this molecule has shown antiproliferative effects, such as tumor growth inhibition on human pancreatic cell lines and mice treated by Frondoside A (Liang et al. 2022). This molecule has also shown anticancer activity with the inhibition of lung cancer and breast cancer cell proliferation with administered doses of 0.01 to 5 μM. It also showed anti-angiogenic effects and significant inhibition of metastasis.
Moreover, this molecule has shown strong immunomodulatory effects and this has a high potential as immunostimulatory agent, particularly with Cucumarioside A2 -2. Therefore, frondoside A may provide curative and prophylactic treatments against diseases which affects the immune status and contributes to the pathological processes (Hossain et al. 2020).

2.3. Phenolic compounds

Due to the absorption of phenolics from phytoplankton, marine invertebrates may possibly serve as a rich source of phenolics. Common types include flavonoids, anthocyanidins, anthocyanins, and tannins, each with distinct chemical structures and bioactivities.
It has been reported that the different body parts (muscles, gonads, digestive tract, and respiratory apparatus) of *C. frondosa* contain a significant amount of phenolics (22.5 to 236.0 mg gallic acid equivalents (GAE)/100 g dw) and flavonoids (2.9 to 59.8 mg of rutin equivalents/ 100 g dw). The highest level of phenolics was obtained from the digestive tract. Three types of phenolic compounds are present in *C.frondosa* : free, esterified and insoluble-bound phenolics (Hossain et al. 2020).
Flavonoids from marine organisms can be used as bioactive compounds, offering diverse potential applications due to their structural diversity that contributes to their unique properties. Thanks to these, they are known to have antioxidant properties including anti-aging and protection against chronic diseases, anti-inflammatory effects, anticancer potential, cardioprotective and neuroprotective activities (Hossain et al. 2020).
Some studies on *C.frondosa* have already shown the antioxidant capacities of extract from the digestive tract. The results have proved that the antioxidant activity of these extracts was directly related to the amount of flavonoids. So Flavonoids are suggested to be mainly responsible for observed activities and can show that it can be used as useful sources of antioxidants for human consumption (Mamelona et al. 2007).

2.4. Lipids

Lipids are present in a large variety in sea cucumbers and offer some biological activities. The gonad and muscle tissues had a significantly higher amount of lipid and fatty acids compared to other body parts. Viscera is the most rich region in polyunsaturated fatty acid (PUFA) (Hossain et al. 2020).
One of the most important lipids present in sea cucumbers is EPA (a Ω3 fatty acid) which has the property to protect cells from oxidative stress and to limit diabetes (Gianasi et al. 2021). This lipid is present in different forms and can be associated with other molecules, such as

with phospholipids and phosphatidylcholine. EPA-Enriched Phospholipids from the sea cucumber suppressed lipid accumulation and lipid droplets (LDs) in liver and adipose tissue by inhibiting the LD associated protein FSP27 in a high-sucrose diet mouse model (Gianasi et al. 2021). They have also shown a neuroprotective antioxidant activity. In fact, in vivo studies reported prevention of the development of learning and memory impairments and the preceding in vitro studies on cells confirmed antioxidant activity in the H_2O_2 oxidative-stress induced cells.
Carotenoids are a diverse group of lipophilic pigments and the major component of carotenoids in *C.frondosa* is cucumariaxanthin (Zakharenko et al. 2020). The second main carotenoid was canthaxantin, before Cucumariaxantin B and lutein (Zakharenko et al. 2020).

2.5. Proteins and peptides

Collagen is reported to be the major protein of sea cucumber with approximately 70% of insoluble collagen fibrils present in the body wall, while the crude protein in dried sea cucumber estimated around 83% of its dry weight. The most abundant collagen is the type I of collagen and cucumber, which is very thermally stable (Hossain et al. 2020). This protein have showed various activities like antihypertensive and anti-aging activity.

Sea cucumber peptides (SCP) were reported to improve memory activity in an amnestic mouse model and to have antimicrobial activity (Gianasi et al. 2021).
Firstly, on one hand, all the doses (650, 1300 and 2600 mg/kg of SCP) significantly reduced the latency phase and shock time, so transmission of signals and on other hand, it permitted a increasing of the acetylcholine content and acetylcholinesterase activity.
Secondly, the extracts of sea cucumber have shown potential antimicrobial activities due to the presence of several crucial enzymes, mainly against the Gram-positive bacteria, suggesting that marine echinoderms, particularly *C. frondosa* are a possible source for the discovery of novel antibiotics. Moreover, a novel antimicrobial peptide from this organism has the property to have a broad range of antimicrobial activity against both Gram-positive and Gram-negative bacteria.

3. Valorization processes of co-products of sea cucumbers

The next table presents what has been done already to upgrade sea cucumbers.

Type of molecule	Polysaccharides (FCS and fucoidans)	Proteins	Saponins	Phenolic compounds	Lipids
Conventional extraction processes	Chemical hydrolysis (can be followed by an enzymatic hydrolysis)	Chemical hydrolysis	Chemical hydrolysis	Heat assisted / percolation / Soxhlet	Organic solvent
Eco-friendly mechanism of extraction	High Hydrostatic Pressure : HHP Enzymatic extraction : EAE	Enzymatic extraction EAE Ultrasound Assisted Extraction Pressurized Liquid Extraction	Supercritical Fluid Extraction : SFE	Ultrasound-assisted extraction : UAE	Supercritical Fluid Extraction : SFE with CO2

Table 2 : Summary of conventional and eco-friendly mechanisms of extraction of each type of by-products

Sea cucumbers can be valorized for medicinal applications, due to the diverse biological activities of molecules found in the cell wall muscle, viscera, gonad, digestive tract, tentacles and internal organs. Currently, the cell wall of the sea cucumber is the major consumed part for nutrition. However, a large part of the cell wall is discarded and other regions of the body of this organism, such as the viscera, gonad, digestive tract and tentacles. These regions represent by-products which can be valorized for health applications.

After the consumption of the body wall, regions of the organism can be valorized by different eco-friendly processes of extraction. Precedent studies showed that:

Enzyme Assisted Extraction can be the most adapted process to extract polysaccharides and proteins of the body wall. Glucosidases and proteases can be used to disrupt links between molecules.

Ultrasound Assisted Extraction could be the most adapted method for the extraction of phenolic components found in digestive tract, tentacles and internal organs

Supercritical Fluid Extraction is the better process for the extraction of lipids contained in muscles and gonads.

Sea cucumber is a sustainable resource, widely available in Northern countries. This organism is rich in molecules and represents an impressive diverse profile. Due to their large variety in molecules, this cucumber presents several bioactive molecules of interest for medicine and health.

Indeed, their composition in polysaccharides (FCS and fucoidans), proteins (amino acids and bioactive peptides or proteins), saponins (particularly Frondoside A), lipids and nutrients allow various activities such as an antimicrobial activity, an antidiabetic activity, antidiabetic activity, anticancer activity, anti-inflammatory activity and anti-hypertensive activity.

Presently, eco-friendly mechanisms of extraction are in development and expanding for use on an industrial scale, due to their low impact on the environment and our health, in comparison to conventional extraction processes.

The cultivation of the sea cucumber is easy and feasible in IMTA systems (Integrated Multi-Trophic Aquaculture) and the co-cultivation of H. forskali and H. tubulosa with oysters, shrimps, algaes may show that this organism not only can ingest but also assimilate the organic waste of effluent water. So, it's a promising source for bioremediation.

It can possibly be used to limit and reduce the contamination of the natural environment due to aquaculture. It's a contribution to environmental sustainability and bioremediation and it's a natural resource which would enable the sustainable development of aquaculture.

The sea cucumber represents a promising available and sustainable natural resource with high medicinal properties and a potential for industry with the development and the use of eco-friendly mechanisms of valorization. A variety of by-products can largely be valorized with these processes.

In this study, we are focusing on *Holothuria forskali* and *H. tubulosa*. These species can become interesting to cultivate in order to anticipate climate change that could affect shrimp and oysters currently produced in Charente Maritime. Indeed, their production condition are submitted to water evolution (in term of temperature and salinity). Introducing a new specie can be off interest. Sea cucumbers are already widely used marine organisms but mostly in Asia continent. The objectives of this project are to characterise them to understand their qualities in case they are cultivable here in Charente-Maritime, France.

III. PROXIMAL CHARACTERISATION OF SEA CUCUMBERS

Sea cucumbers were collected in mars 2023 from swamps of Oleron Island (F-17) (2 units of *H. tubulosa*) and april 2024 thanks to Leila Parizadeh (8 units of *H. forskali*). They were killed, skin was separated from viscera and they were freezed.
Optimisation of assays were done using *H. tubulosa*. All results of this report were obtained with *H. forskali* due to the low number of *H. tubulosa*.

1. Cleaning/Washing and Separation

The procedure started with washing the sea cucumbers and separating their inner parts (figure 3), cutting them in pieces.

Figure 3 Washed and cut sea cucumber (H. Forskali) skins

2. Dry weight measurement

The extractions were applied to the skin of the sea cucumbers.
The dry weight of sea cucumber skin was measured on 10 samples. Different skin samples were placed on an aluminum foil and put into an oven for 24 hours at 90°C. It was found to reach 10,77%±0,78 for our first batch (fresh animals).
Freezed animals were unfrozen and cut into small pieces. Pieces were also submitted to dry weight measurement.
The dry matter was lower for un frozen pieces, reaching only 6,36%.
The total working wet weight was measured as 671,2 g.

3. Ash calculation

The ash percentage of the sea cucumbers was measured with 6 different samples placed in a crucible and put into the oven at 600 degrees for 4 hours (Mufle oven). The ash % was found to be 3,20 on average.

4. The total nitrogen measurement using Kjeldahl method

The Kjeldahl device is used for the analysis of total nitrogen content within the sample. The protein conversion coefficient was 6.25.

Figure 4: Kjeldhal apparatus

The table below presents what was done and results obtained.

	Sample (g)	HCl volume (ml)	Reaction yield (%)	N (g)/100g sample	Protein content %/ mass	Corrected value (by urea recovery yield)
control		0,6	-	-	Coefficient applied: 6,25	-
urea	0,037	4,67	93,3	-	-	-
Untreated dried skin 1	0,231	8,35		10,63	66,42	70,85
Untreated dried skin 2	0,239	8,35		10,27	64,19	68,48
Untreated dried skin 3	0,222	7,5		9,72	60,73	64,78
Untreated dried skin 4	0,333	12,22		11,44	71,51	76,28

So, as expected again, the sea cucumber skin contained protein, around 70%. The conversion coefficient applied was 6.25. This coefficient may be not adapted to the conversion of nitrogen content in marine species since it was defined as a value used for terrestrial proteins. For example, for algaes, the conversion coefficient is 5.25.

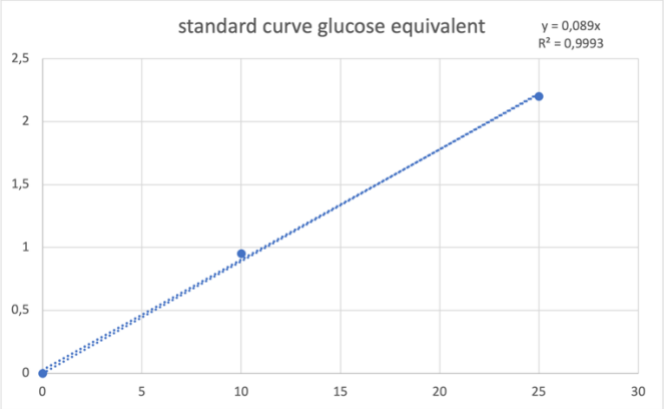
Another Kjeldahl analysis was done after the collagen extraction procedure to compare the nitrogen content between “before extraction” and “after extraction” of proteins. As a result, before-extraction skin contained the highest percentage of nitrogen indicating that some proteins were extracted as expected.

After acetic acid extraction, the remaining protein % was 22±1; after lactic acid extraction, the value was 65%±1 and after HCl extraction, the protein % was 62%±1,5.

Lactic acid extraction yield was really poor and we decided to continue only with acetic acid for tentative collagen extraction.

5. Dubois Total Sugar Analysis

Dubois method has been conducted to measure the total sugar content of the sea cucumbers skins. The standard curve has been prepared with glucose solution. After creating the standard curve, the total sugar content was measured within the samples. A total of 0,471 g of dried sea cucumber skin was weighed and 2 ml of water was poured to make a mixture carefully vortexed. Only the soluble part was analysed.



Dubois Standard Curve (OD340nm vs glucose quantity (µg))

Almost no sugars are presents in the sea cucumber skin since only 0,25% of reducing sugar were measured in the soluble part of the skin. This is quite logical since polysaccharides are tightly linked with other compounds. They are not soluble.

Sulfuric acid digestion is one on the solution to destroy all tissues and measure the osidic compounds.

6. Ethanolic lipid extraction

Lipids are removed usually using solvent and dosed. For this part, the washed sea cucumbers skins were put in pure ethanol and left for 2 hours (picture below). This way, lipids were removed from the sea cucumber skin. After two hours, to determine their lipid concentration, if there is any, the weight of 6 test tubes is measured and 1000 µl of ethanol solution containing extracted lipids is poured into each of them and placed in the oven at 90 degrees for 24 hours. After 24 hours, the lipid percentage has been calculated as 0,68% in mean.

Sea cucumbers in pure ethanol for delipidation

The ethanol-treated sea cucumbers were weighted (442,3 g) then put in 0,1 M NaOH solution and left there for 24 hours. This way the non-collagenous proteins were removed from the skin. After 24 hours the sea cucumbers have been taken out.

Sample of the NaOH solution has been poured into two bottles and placed into the freezer to be freeze-dried.

The remaining solution is placed under a pH meter and recorded at 12,97.

The HCl has been added to decrease the pH, to see if any soluble proteins can precipitate under pH modification. After some HCl addition, precipitation is observed and the colour of the solution has changed. The final pH is decreased to 2,33.

This solution was collected in two bottles and 20 ml of it was poured into a falcon tube to be freeze-dried.

Sea cucumbers in 0,1 M of NaOH

Non-soluble protein observation in acid

Reversible protein solubilisation has been observed when the pH is increased again.

7. Extraction of Collagen

7.1. Extraction procedure

The sea cucumbers are divided into 3 samples of around 140g and placed in different beakers. Each of them had different acids, Acetic Acid (0,5M), Lactic Acid (0,5M), and HCl (0,5M) to start collagen extraction.

Sea cucumbers skins in 3 different acids

After 72 hours, there was no observation of change in the color, so a second extraction was conducted.

After the collection of the first extraction solutions, sea cucumbers are put in the acids again and left there for more 72 hours. For the first extraction solutions, protein determination assays were conducted.

8. The Lowry Protein Assay: soluble protein measurement

The determination of the protein content has been measured using the Lowry Protein Assay. For the Lowry reagent, solutions 1, 2 and 3 have been prepared and the standard curve has been prepared with 3 different proteins: Bovine serum albumin, bovine gelatin and fish gelatin to select the best protein to use as standard.

To prepare the standard curves, 5 different concentrations of protein solutions were prepared, and measured with a spectrophotometer at 700 nm.

Solution 1: Na_2CO_3 (5 mg/ml) plus 1 g of NaOH and adjusted to 250 ml.

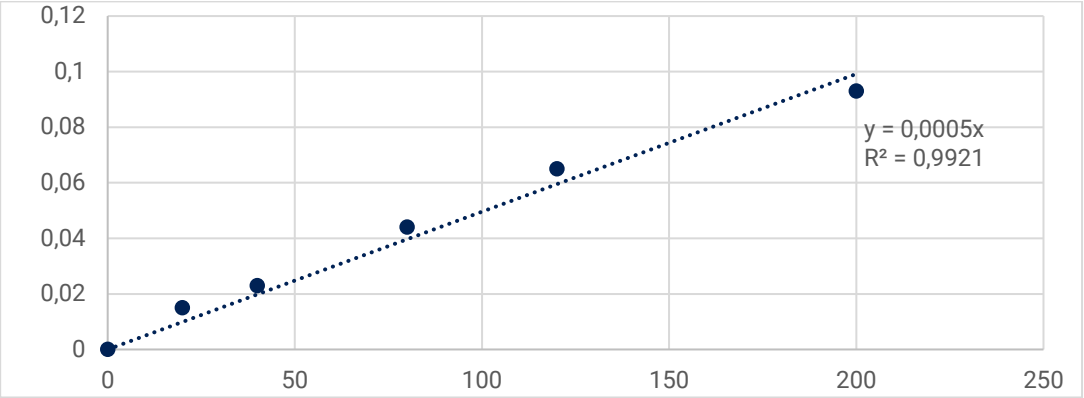
Solution 2: $CuSO_4 \cdot 5H_2O$ solution composed of 250 mg/ml.
Solution 3: 500 mg/40 ml Potassium sodium tartrate tetra-hydrate ($C_4H_4KNaO_6$)

Solutions 1,2 and 3 for the lowry protein assay.

For the lowry reagent, 50 ml of solution 1, 1 ml of solution 2 and 1 ml of solution have been mixed.

The To 1ml of protein solution, 2ml of Lowry solution are added and allowed to stand for 10min. 200µL of Folin reagent is then added and allowed to stand for 30min. The OD à 700nm is then read on the spectrophotometer.

We eventually selected the fish gelatin to measure the content of soluble protein because of its good interaction with the reagents and its similarity with sea cucumber protein (presumed to be gelatin)

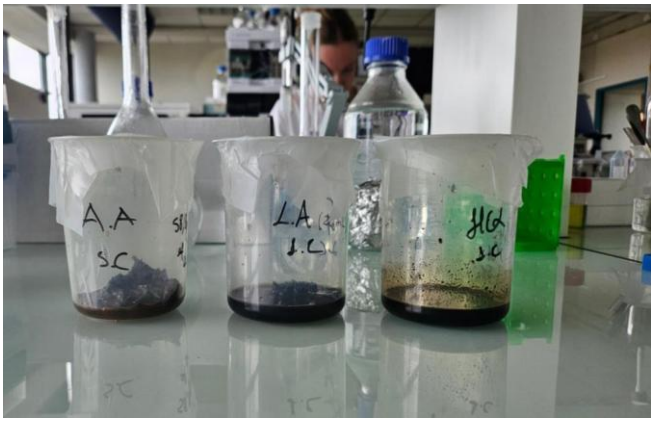


Standard Curve of Fish Skin gelatin for soluble protein assay

After creating the standard curve, the first extraction solutions were analysed. Before analysing with the Folin Reagent, the pH of the solutions is adjusted to neutral pH with NaOH.

From HCL extraction, the soluble protein concentration was 17,42g/L. For lactic acid, the protein concentration was 6,09g/L and for acetic acid, 7,09g/L.

The results indicated that HCl, the strongest acid, has extracted more proteins than any other acids, however, the higher protein concentration doesn't indicate that all the proteins are collagen. Moreover, the observation after the first extraction, there was no colour change in the solutions indicating little extraction, so another 1:3 ratio of acids was poured onto the sea cucumbers again.



Second Extraction of collagen with HCl, lactic and acetic acid.

The Lactic Acid Sea Cucumber mass after the first extraction: 50,5 g. The Acetic Acid Sea Cucumber mass after the first extraction: 58,8 g. The HCl Sea Cucumber mass after the first extraction: 49,4 g.

The first extractions and the second extraction solutions were freeze-dried. 150 ml of each solution are poured into three bottles to be freeze-dried. Another Lowry Protein assay was conducted with the collected extraction powders presented on the picture below.

Freeze-dried samples after extractions

Powders were quite brown indicating a high content in pigments that were extracted together with proteins.

For HCL, lactic and acetic acid, soluble protein percentage of powders obtained were respectively 55; 20 and 9%. The strong acid is able to extract more proteins but the brown color of the powder indicate that they may be denatured because combined with pigments.
Moreover, the structure and the colour of the first freeze-dried sample of lactic acid solution were promising to have the potential to be collagen.

9. Electrophoresis analysis

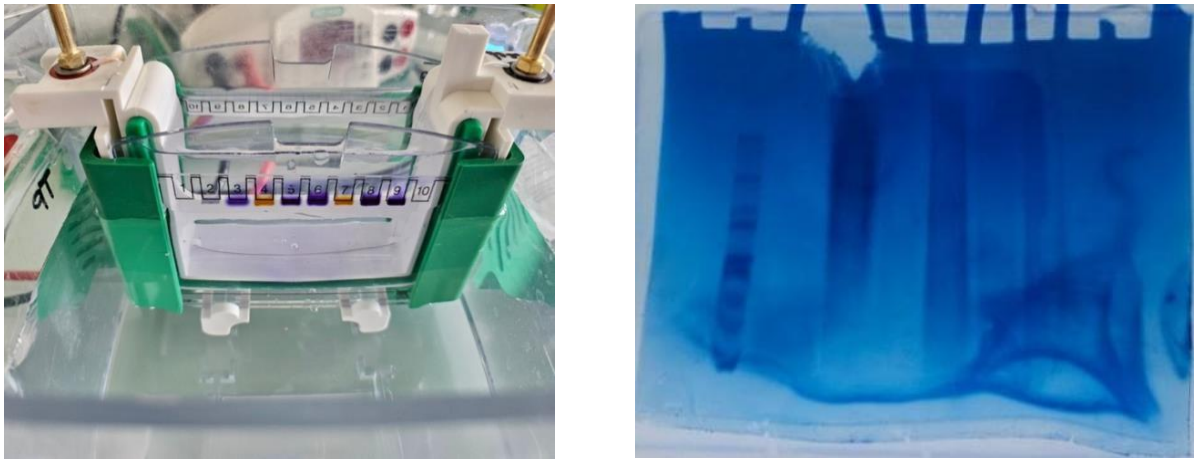
Electrophoresis has been conducted to examine which proteins are in the extracted samples. Each concentrated solution has been put on the electro-gel to be identified by size and charge. The gel was a precasted gel from 4 to 20% acrylamide from Biorad. The molecular weights markers were alos from Biorad and covered a range from 10 to 250kDa.Gel were coloured using Coomasie Blue solution.

A total of 4 electrophoresis were conducted. On the gel, the wells from the 3rd until the 9th are used, in total of 7 samples. The first and the 10th gaps have been left empty and the second gap has been put with the molecular weight marker.

The first one contained the samples of respectively, HCl first extraction powder (50mg/0,5ml), Lactic Acid second extraction powder (55mg/0,5ml), uncentrifuged and untreated lactic acid solution first extraction powder, centrifuged untreated lactic acid solution first extraction powder, uncentrifuged, untreated acetic acid solution first extraction powder and centrifuged and untreated acetic acid solution first extraction powder, nothing has been put on the 9th gap. The results indicated that, only in HCl first extraction powder contained collagen, however, the concentration was too much. The second electrophoresis contained the samples of; HCl first extraction powder (diluted to 50 mg/ml), Lactic acid freeze-dried sample (54mg/ml), Lactic acid 2nd extraction powder (diluted to 55mg/ml), Lactic acid 1st extraction powder (50mg/ml), Acetic acid 1st extraction freeze-dried sample (52mg/ml) and Acetic acid 2nd extraction powder (49mg/ml). The results indicated that all the samples contained collagen. Still, the concentration was insufficient to see clearly on the gel, so for the third gel the concentration of Lactic acid freeze-dried (105mg/ml), Acetic acid 2nd extraction powder (75mg/ml) was increased and conducted again as well as with non-collagenous protein solution (107 mg/ml) and acetic acid 2nd extraction solution (104 mg/ml). The results indicated there

was no collagen inside the non-collagenous protein solution, but the other ones had it. Also, another gel has been conducted with the sample of collagen coming from the sturgeon fish skin to compare on the gel.

As a result, the protein solutions of HCl, and Lactic acid 1st extraction freeze-dried sample contained collagen.



Electrophoresis apparatus and obtained gel.

Lactic acid extraction (well 4) showed intense blue bands indicating the presence of protein of quite high molecular weights resembling to molecular mass expected to be those of beta-chain collagen (200kDa) and alpha-chain collagen (100kDa).

This first extraction procedure allowed us to obtain brown powders containing proteins that could be collagen.

10. Collagen extraction II

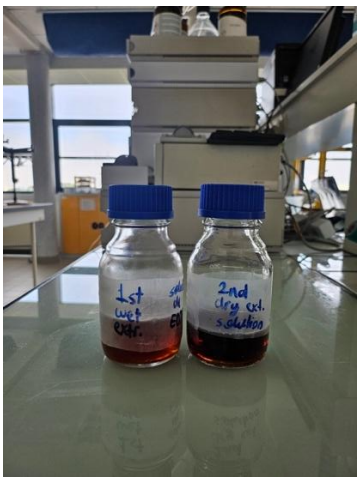
Another extraction was performed in autumn 2024. Since the drying of sea cucumber could be a costly step, so, others experiments were conducted to determine of this step is really crucial.

The wet weight was measured as 240gr in total. Sea cucumbers were equally divided into two parts: one for the collagen extraction from wet skin and dry extraction. For the dry skin extraction sea cucumbers were put in oven at 90°C for 1 day, until the weight stabilised.

For each of the two groups, 3 samples have been made to calculate the dry weight. The average dry weight of the wet sea cucumbers is found 5,53%, close the the value obtained previously (6%) while for the dry sea cucumbers it has been found 83,66% which is logical since the skin was dried.

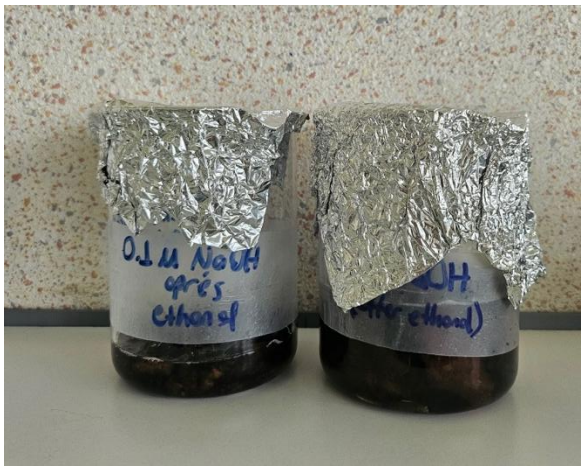
0,5 M of NaCl + 50 mM of EDTA has been prepared to break down the collagen fibrils to promote collagen extraction process.

Both groups of the sea cucumbers are treated with this solution (1:1) and left for 3 days at 4 °C while gently stirring. After three days, solutions are collected and the filtered supernatant centrifuged at 7500 rpm for 30 minutes and freezed directly to analyse the protein content. The remaining sea cucumbers skins are put in pure ethanol (1:1) and left it for a day at room temperature to clean from the lipids.

Filtered sea cucumbers on paper filter and the collected solutions

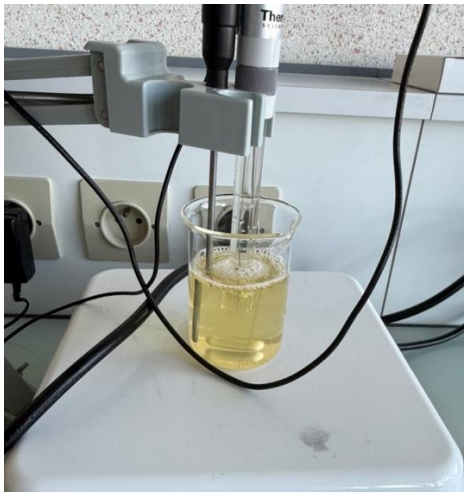
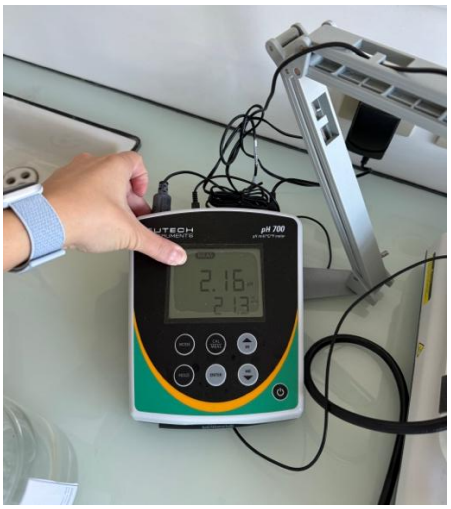
24 hours after ethanol cleaning, the sea cucumbers are put in 0,1 M of NaOH (1:3) to clean from non-collagenous proteins. Skins were left in the solutions for 3 days at 4 degrees while gently stirring.



Sea cucumbers in 0,1M of NaOH after Ethanol

The lipid percentage was calculated after the cleaning part with ethanol. For each group, six different samples has been prepared and the average lipid percentage for the wet sea cucumbers has been found 1,03% while for the dry sea cucumbers this was 2,03% .

The pH of the NaOH solutions is observed as 11,75 in the beginning. HCl has been added to observe the reversible precipitation of proteins by changing the pH. However, no precipitation has been observed, concluded that there was no reversible protein fibrils inside the solution. This may be due to skin alteration after longer storage at -18°C.

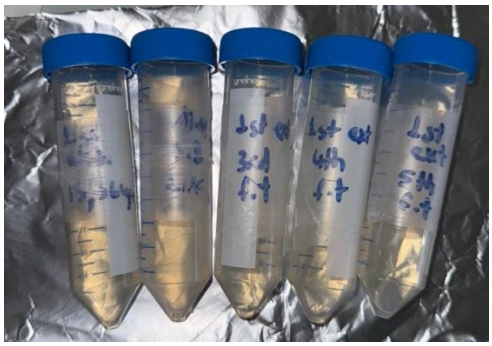
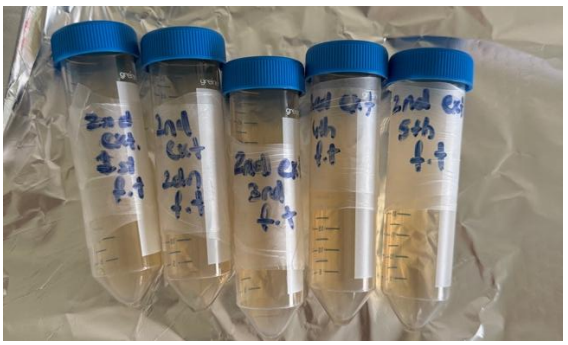
The pH determination of 0,1M NaOH solution and adjusting it to pH 2,16

After 3 days of NaOH treating, sea cucumbers are put in 0,5 M of Acetic Acid (1:4) to extract collagen.

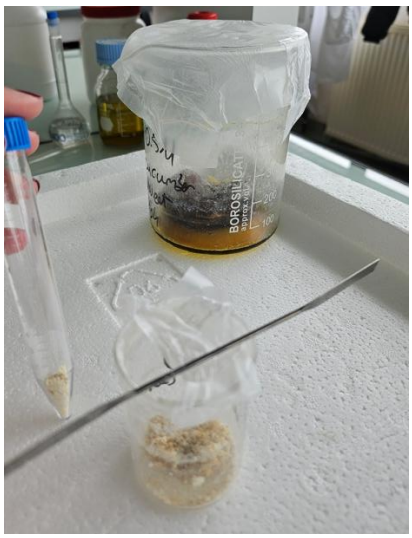
The sea cucumbers are left in solutions for three days at 4 degrees.

After three days, the supernatants are filtered and poured into falcon tubes to be freeze-dried.

Sea cucumbers in acetic acids

Extraction solutions in falcon tubes to be freeze-dried

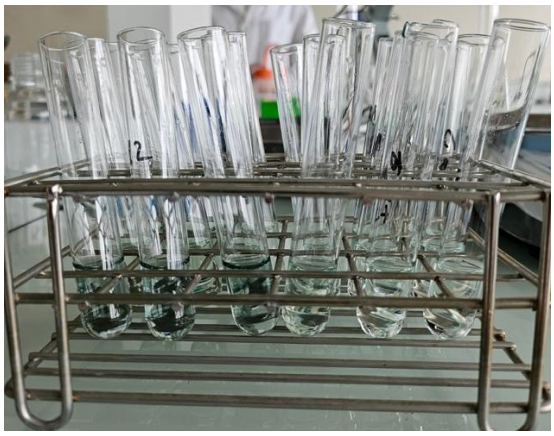


After the extraction, the collected powder of freeze-dried solution

The powder obtained after freeze drying was less colours than the previous one. The NaOH +EDTA washing solution was more efficient to remove non collagenous proteins and may be also pigments.

After creating the standard curve for a now soluble protein assay measurement, the first extraction powder (coming from wet extraction) was analysed. The sample has a concentration powder of 10mg/ml.

For the analysis, 300 microlitre of sample has been completed to 1 ml with water. 2 ml of Lowry Reagent has been put and waited for 10 minutes. After that, 200 microlitres of Folin reagent has been put and waited for 30 minutes. After 30 minutes the samples (picture below) were analysed reading the OD at 700 nm on a spectrophotometer.

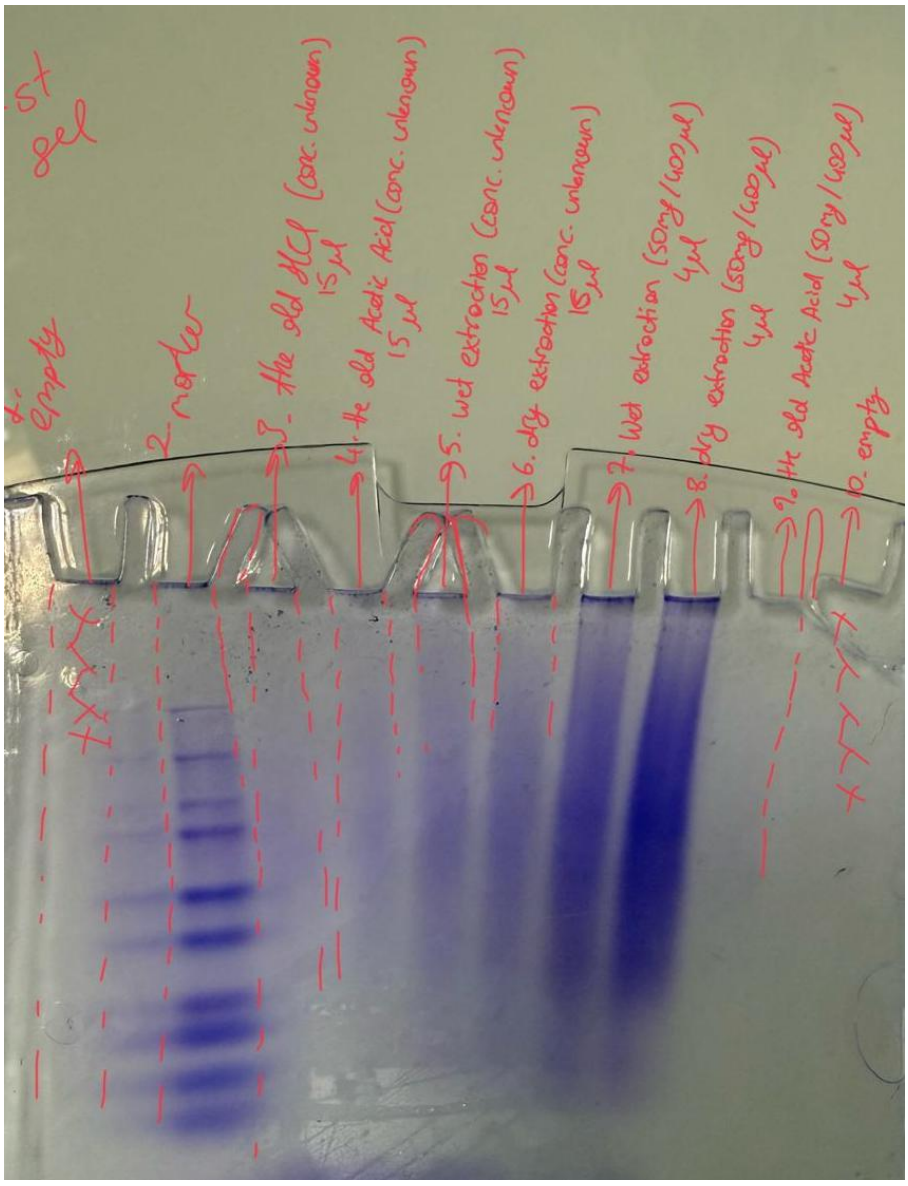


The same method has been done for the second dry extraction powder with a concentration solution of 11,5mg/ml.

As expected, the protein percentage in the powder obtained after extraction protein from dry skin was found almost two times higher. Indeed, The protein powder extracted from wet skin was of 16% whereas the protein content in the powder extracted from dry skin was 34%

Electrophoresis has been conducted to examine which proteins were extracted. Each concentrated solution has been put on the gel to be identified by size and charge. Four different protein solutions

have been made. HCl and Acetic Acid extraction powders have been put in the electrophoresis analysis as well. All solutions have the concentration of 20 mg/ml. In total of 2 SDS-PAGE gels are prepared, in one gel the total amount of samples were 25µL per well.
As observed on the picture below, the HCL extracted powder present few bands. All proteins extracted are in too small concentration to be visible on the gel or they are too small (degraded into peptides). This is also coherent with the previous observation that this extract contain nitrogen but few proteins.
Wet extraction gave also a powder with less visible proteins. We can observe that after the extraction of protein from dry skin, blue colour remained. This indicated the presence of protein of a wide molecular weight range.
Collagen extract was not present under expected bands but under diffuse bands indicating a wide molecular weight distribution.



IV. CONCLUSION

From all this word, we demontrated that sea cucumber collected from Charente Maritime contained manly protein (up to 70%) for the skin part and minor content of other macromolecules (between 3-4% mineral, less than 1% osidic compounds and lipids).
Collagen extraction gave interesting results since high molecular weight protein were obtained but the acid used may have degraded the fibrils.

The heavy metal content was not measured but it is known that sea cucumber can accumulate such compounds. In case of further valorisation, their concentration must be assessed. However, if sea cucumber are grown in controlled environment as expected, then this contamination will be drastically reduced.

The viscera are still under investigation to analyse their proximal content. They eventually could be used to produce peptide by autolysis as it is done in other works.

V. BIBLIOGRAPHY

Gianasi BL, Hamel J-F, Montgomery EM, Sun J, Mercier A. 2021. Current Knowledge on the Biology, Ecology, and Commercial Exploitation of the Sea Cucumber Cucumaria frondosa. Reviews in Fisheries Science & Aquaculture. 29(4):582–653. https://doi.org/10.1080/23308249.2020.1839015

Hossain A, Dave D, Shahidi F. 2020. Northern Sea Cucumber (Cucumaria frondosa): A Potential Candidate for Functional Food, Nutraceutical, and Pharmaceutical Sector. Marine Drugs. 18(5):274. https://doi.org/10.3390/md18050274

Janakiram NB, Mohammed A, Rao CV. 2015. Sea Cucumbers Metabolites as Potent Anti-Cancer Agents. Mar Drugs. 13(5):2909–2923. https://doi.org/10.3390/md13052909

Liang Q, Ahmed F, Zhang M, Sperou N, Franco CMM, Feng Q, Zhang W. 2022. In Vivo and Clinical Studies of Sea Cucumber-Derived Bioactives for Human Health and Nutrition From 2012-2021. Frontiers in Marine Science [Internet]. [accessed 2023 Nov 14] 9. https://www.frontiersin.org/articles/10.3389/fmars.2022.917857

J. Mamelona, É. Pelletier, K. Girard-Lalancette, J. Legault, S. Karboune, S. Kermasha
Quantification of phenolic contents and antioxidant capacity of Atlantic sea cucumber, Cucumaria frondosa Food Chemistry, 104 (3) (2007), pp. 1040-1047, 10.1016/j.foodchem.2007.01.016

Nelson EJ, MacDonald BA, Robinson SMC. 2012. A Review of the Northern Sea Cucumber Cucumaria frondosa (Gunnerus, 1767) as a Potential Aquaculture Species. Reviews in Fisheries Science. 20(4):212–219. https://doi.org/10.1080/10641262.2012.719043

Ramalho A, Leblanc N, Fortin M-G, Marette A, Tchernof A, Jacques H. 2020. Characterization of a Coproduct from the Sea Cucumber Cucumaria frondosa and Its Effects on Visceral Adipocyte Size in Male Wistar Rats. Marine Drugs. 18(11):530. https://doi.org/10.3390/md18110530

Song Z, Zhu M, Wu J, Yu T, Chen Y, Ye X, Li S, Xu N. 2022. Fucoidans from Cucumaria frondosa ameliorate renal interstitial fibrosis via inhibition of the PI3K/Akt/NF-κB signaling pathway. Food Funct. 13(3):1168–1179. https://doi.org/10.1039/D1FO03067A

Wang Y, Wang J, Zhao Y, Hu S, Shi D, Xue C. 2016. Fucoidan from sea cucumber Cucumaria frondosa exhibits anti-hyperglycemic effects in insulin resistant mice via activating the PI3K/PKB pathway and GLUT4. J Biosci Bioeng. 121(1):36–42. https://doi.org/10.1016/j.jbiosc.2015.05.012

Zakharenko A, Romanchenko D, Thinh PD, Pikula K, Hang CTT, Yuan W, Xia X, Chaika V, Chernyshev V, Zakharenko S, et al. 2020. Features and Advantages of Supercritical CO2 Extraction of Sea Cucumber Cucumaria frondosa japonica Semper, 1868. Molecules. 25(18):4088. https://doi.org/10.3390/molecules251840

VI. Annexe 2

Gebré DECHASSA, Juliette SMITH (2024) Valorisation des vers tubes par le dosage et l'identification des protéines et sucres les composants. Rapport de stage, BCBS team, LIENSs, La Rochelle Université. Supervision Stéphanie Bordenave-Juchereau

Valorisation des vers tubes par le dosage et l'identification des protéines et sucres les composants.

Dechassa Gebré, Mai 2023
Juliette Smith, Mai-Juin 2024

BCBS Team
Biotechnologie et Chimie des Bioressources pour la Santé
External Supervisor: **Stéphanie Bordenave-Juchereau**
stephanie.bordenave@univ-lr.fr

I. Matériels et Méthodes

Trois échantillons distincts ont été analysés :
Les tubes sales : tubes entourés des coquillages et des fibres
Tubes propres : structure souple débarrassée des fibres et coquillages par un lavage à l'eau
Vers : animaux.

En termes de représentation, si les tubes ayant contenu les vers représentent 100% en masse (humide) alors, les tubes décoquillés manuellement représentent environ 11% en masse et les tubes nettoyés à l'eau courante (dessablés) représentent environ 6% en masse humide. L'image 1 présente les coquilles détachées (à gauche) des tubes (à droite), collectés en mars 2023.

Image 1 : Coquilles détachées des tubes (à gauche) et tubes (à droite).

Les tubes nettoyés contiennent environ 60% d'eau. Il sera donc compliqué de valoriser ces tubes qui deviennent très friables une fois secs et restent « contaminés » avec des particules de vase.
La valorisation des tubes sera surtout une valorisation inorganique des petites coquilles de coques.
Nous nous sommes focalisés sur les tubes car les vers seraient valorisés pour la pêche. Les tubes seraient donc des coproduits de la récolte ou de la production des vers.

II. Résultats

1. Détermination de la masse sèche

Afin d'obtenir des échantillons secs à analyser, nous les avons placés dans une étuve réglée à 90°C. Le temps passé à l'étuve varie de 3h pour les tubes à 48h pour les vers.

Echantillon	tubes sales	tubes propres	vers
Pourcentage de masse sèche	29 % (±0,1)	28% (±0,3)	23% (±0,3)

Le pourcentage de masse sèche des tubes propres et sales est très proche, ce qui peut se comprendre par le fait que les tubes sales sont entourés de coquilles et de sable. Ces derniers ne stockant pas d'eau, la masse sèche correspond essentiellement au tube en dessous et donc égale à celle des tubes propres.
Les vers contiennent environ 23% de matière sèche.

2. Dosage des cendres

Préparation des échantillons préalablement séchés à l'étuve à 90°C puis pesée des échantillons avec une balance de précision dans des creusets.
Utilisation d'un four à moufles réglé à 500°C pendant 3 à 4h.
Les résultats sont présentés ci-dessous, tableau 1.

Echantillon	Tubes sales	Tubes propres	Vers
Moyenne % cendres sur matière sèche	79,8±8,5	43,2±3	30,7±7

Tableau 1 : Tableau récapitulatif du taux de cendres pour les trois échantillons.

Le taux élevé de cendres pour les tubes sales pourrait correspondre pour une grande partie aux coquillages entourant le tube. Ces minéraux pourraient être valorisés comme amendement sur les sols après décomposition de la matière organique. Les vers contiennent environ 30% de minéraux.

3. Dosage de l'azote total

Le taux en azote a été obtenu grâce à la méthode de Kjeldahl à partir d'échantillons secs de tubes sales ; tubes propres ; coquilles entourant les tubes sales et vers.
La quantité d'azote a pu être convertie en quantité de protéines en utilisant le facteur de Jones (soit 6,25).
Il apparait que les tubes sales secs contiennent environ 3% de protéines alors que les tubes propres secs environ 15%. Si une valorisation des protéines est envisagée, il conviendra de nettoyer les tubes des algues et coquilles qui les encombrent.
Les vers contiennent, quant à eux, environ 14% de protéines sur masse sèche.
Aucune présence notable de protéines n'a été détectée dans les coquilles entourant les tubes sales.
Il est à noter que le dosage de Kjeldahl utilise le facteur de conversion de 6,25. Le facteur spécifique à appliquer aux ressources marine n'est pas documenté mais est certainement plus faible ce qui conduira à une diminution de la quantité de protéines mesurée via cette méthode.

4. Dosage des protéines solubles selon Lowry

La valorisation des protéines des échantillons vers, tubes propres et tubes sales nécessite leur solubilisation préalable.
Les tubes ne sont pas solubles dans l'eau ni dans l'éthanol, même à chaud.
Une solubilisation à l'acide fort concentré a été testée.
Deux solutions ont été créées, contenant chacune du tube propre et sec de masse environ égale à 300 mg. Dans un échantillon, 50 mL de HCl 3M ont été versés et, dans l'autre, 50 mL de HCl 6M.
→ Concentration de départ des deux échantillons : 6mg/mL
Ces deux échantillons représentent les solutions mères pour les dosages de protéines à venir.

4.1. Cinétique de libération des protéines de tube dans l'acide

Immersion de 2 tubes de +/- 300 mg chacun dans 2 béchers contenant 2 solutions de 50 mL d'HCl de concentration 6M et 3M. Soit une concentration de 6 g/L. Température ambiante.
Prélever 500 µL de solution et ajouter 500 µL de NaOH 6M afin de neutraliser la solution pour que les réactifs de Lowry et de Folin puissent agir.
Les résultats de cette cinétique de libération des protéines solubles est présenté dans le tableau suivant :

	Concentration en protéines solubilisées (µg/µL) Solution de départ 6µg/µL en matière sèche)	
Temps (h)	HCl 3M	HCl 6M
0	0	0
1	0	0
2	0	0,132
4	0,04	0,386
4,5	0,05	0,612
5	0,062	0,734
5,5	0,154	0,778
6	0,094	0,806 (13,43% de la masse initiale)
17	0,064	0,774

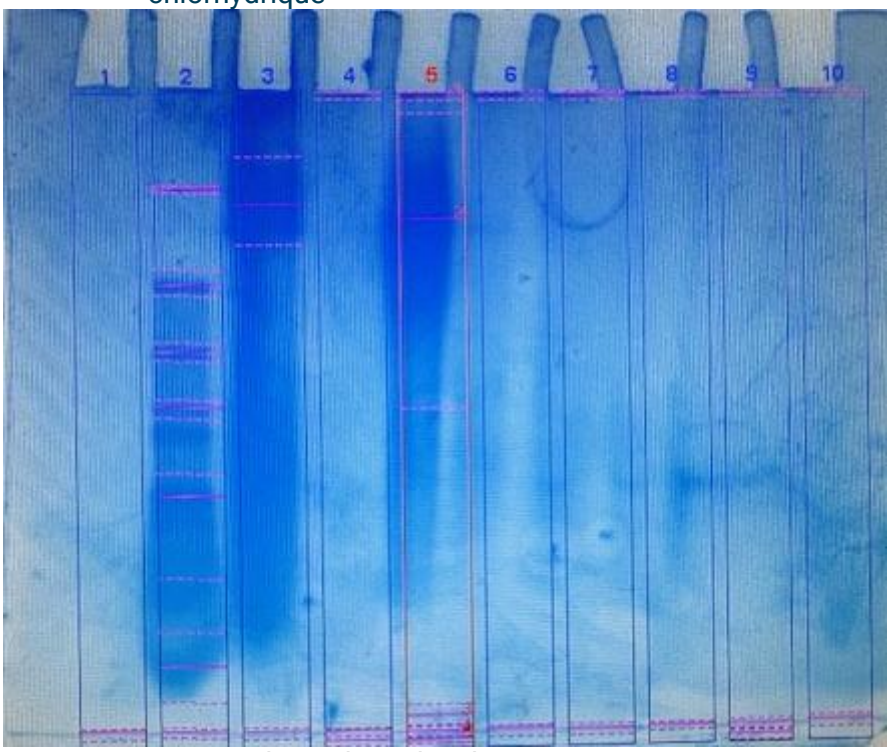
Tableau 2 : Résultats des quantités de protéines dans le prélèvement pour les deux concentrations en HCl selon le temps de prélèvement

Observations :

- Au bout de 45 min d'immersion dans HCl 6M, le tube se met à flotter à la surface et commence à se déliter sous agitation à l'aide d'une spatule.
- Au bout de 2h d'immersion dans HCl 6M, le tube devient friable
- Au bout de 17h d'immersion, les protéines sont dispersées

L'acide chlorhydrique 6M permet de solubiliser le tube et pas celui dosé à 3M.
La concentration en protéine de la solution atteint pour 6h, 0,806µg/µL. Cette valeur représente 13,43% de la masse initiale soit la quasi-totalité des protéines qui ont été dosées par la méthode de Kjeldahl.
Les protéines seraient intégralement solubles après 6h dans l'acide chlorhydrique 6M ce qui semble logique.

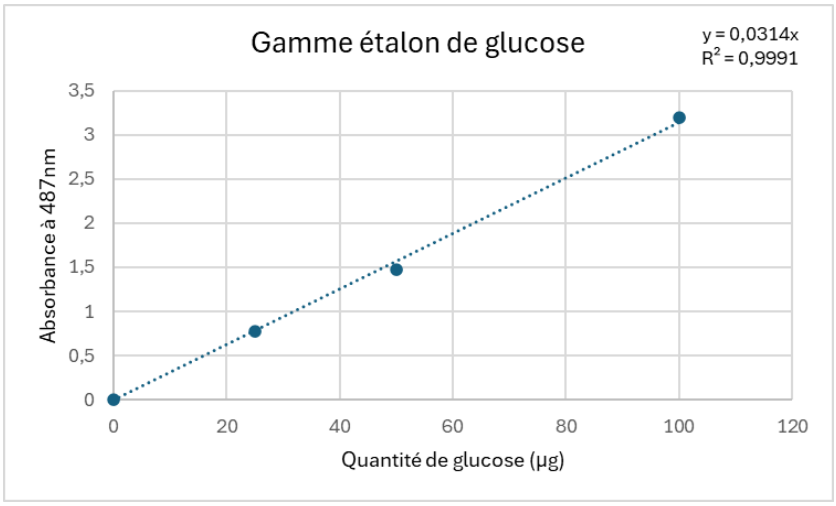
4.2. Analyse électrophorétique des échantillons de tubes solubilisés dans l'acide chlorhydrique



Puits 2, Marqueurs de poids moléculaires
Puits 3 vers broyés 30µg
Puits 5 vers boyés 15µg
Puits 6 à 8 : cinétique de libération des protéines issues des tubes
Seuls les protéines présentes dans les vers sont en quantité suffisante pour être visualisées.

5. Dosage des sucres totaux - Dosage de Dubois

Afin de déterminer et doser la présence de sucres dans les tubes et les vers dans différentes solutions, nous avons réalisé un dosage de Dubois. Nous avons d'abord réalisé une gamme étalon de glucose à partir d'une solution de concentration de 5 g/L. Elle est présentée ci-dessous :



Gamme étalon de solution de glucose à 5 g/L pour le dosage de Dubois

Une solution de concentration massique 16g/L contenant du tube propre (TP) et de l'HCl 6M broyée au potter a été dosée :
Cette solution contient environ 4,2±0,4g/l d'équivalent glucose. Les tubes propres contiendraient donc environ 26% d'équivalent glucose sur poids sec.
La solution obtenue après avoir broyé des vers (1138mg humides/mL (Matière sèche 13%) soit environ 148mg/mL vers secs) a été dosée. Le contenu en équivalent glucose est de 1,4%. Les vers contiennent donc très peu de glucides.

III. Conclusion

En conclusion, l'analyse proximale révèle que les vers tubes contiennent principalement des protéines.
La partie tube reste celle qui est majoritaire en termes de masse. C'est certainement cette partie qui sera à valoriser à l'avenir si la culture des vers tubes se développe.
La partie tube est composée de polysaccharide. Si elle n'est pas agrégée avec des débris qui la contamine, elle pourra, elle aussi, être valorisée.

Ces travaux ont permis de former à et par la recherche deux étudiants : Dechassa Gebre, Master 1 en Biotechnologies, parcours Joint Master Degree in Marine Biotechnology. Juliette Smith, Licence 3 Sciences pour la santé, parcours Biochimie.

Pierrick Barbier
Référent scientifique aquaculture
p.barbier@cape-na.fr

CAPENA – Expertise et Application
Prise de Terdoux 17480 Le Château d'Oléron
T : 05 46 47 51 93
www.cape-na.fr