



# PROMISEANG

Alternative PROteins from Microbial fermentation of non-conventional SEA sources for Next-Generation food, feed and non-food bio-based applications

## D2.6

Features of marine by-products for successful biomass fermentations

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## 1. PROJECT INFORMATION

**Project full title:** Alternative PROteins from Microbial fermentation of non-conventional SEA sources for Next-Generation food, feed and non-food bio-based applications

**Acronym:** PROMISEANG

**Call:** HORIZON-JU-CBE-2022

**Topic:** HORIZON-JU-CBE-2022-R-04 - Proteins from alternative and unconventional sources

**Start date:** 1<sup>st</sup> September 2023

**Duration:** 48 months

**List of participants:**

| Number | Name of beneficiary                                                                                            | Acronym of beneficiary | Country |
|--------|----------------------------------------------------------------------------------------------------------------|------------------------|---------|
| 1      | UNIVERSIDAD DE VIGO                                                                                            | UVIGO                  | ES      |
| 2      | IST-ID ASSOCIACAO DO INSTITUTO SUPERIOR TECNICO PARA A INVESTIGACAO E O DESENVOLVIMENTO                        | IST-ID                 | PT      |
| 3      | MOA BIOTECH SL                                                                                                 | MOA                    | ES      |
| 4      | INSTYTUT BIOTECHNOLOGII PRZEMYSŁU ROLNO-SPOŻYWCZEGO IM PROF WACŁAWA DĄBROWSKIEGO - PAŃSTWOWY INSTYTUT BADAWCZY | IBPRS                  | PL      |
| 5      | CONTACTICA SL                                                                                                  | CTA                    | ES      |
| 6      | CENTIV GMBH                                                                                                    | CENTIV                 | DE      |
| 7      | AGENCIA ESTATAL CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS                                                | CSIC                   | ES      |
| 8      | BIOZON GMBH                                                                                                    | BZN                    | DE      |
| 9      | LABORATORIO IBERICO INTERNACIONAL DE NANOTECNOLOGIA LIN                                                        | INL                    | PT      |
| 10     | CONSERVAS RIANXEIRA SA                                                                                         | JEALSA                 | ES      |
| 11     | ALLATORVOSTUDOMANYI EGYETEM                                                                                    | UVMB                   | HU      |

## 2. DELIVERABLE DETAILS

|                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|----------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Document Number:</b>    | D2.6                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| <b>Document Title:</b>     | Features of marine by-products for successful biomass fermentations                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| <b>Dissemination level</b> | PU - Public                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| <b>Period:</b>             | PR1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| <b>WP:</b>                 | 2                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| <b>Task:</b>               | 2.5                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| <b>Author:</b>             | UVIGO<br><br>Universidade de Vigo                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| <b>Abstract:</b>           | This deliverable aims to present the progress of the project by preselecting the 10 most promising marine biomasses for fermentation. The selection process considers the characterization of the biomasses and market analysis. Four key criteria were established to guide the preselection process, ensuring a comprehensive evaluation. The deliverable provides a thorough overview of the advancements made in identifying viable marine biomasses for further development within the project. |

| Date       | Description                    | Partners involved                                                                                                   | Document version |
|------------|--------------------------------|---------------------------------------------------------------------------------------------------------------------|------------------|
| 05/02/2025 | Draft of deliverable 2.6       | JEALSA – Paula Hermo<br>UVIGO – Alicia Pérez (BIA)<br>UVIGO – Carla Cameselle (AA1)                                 | 1.0              |
| 13/02/2025 | Final draft of Deliverable 2.6 | UVIGO – Alicia Pérez (BIA)<br>UVIGO – Carla Cameselle (AA1)                                                         | 1.1              |
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### 3. INTRODUCTION

Meeting the global protein demand, projected to reach 1.25 billion tons annually by 2050, requires sustainable solutions that go beyond traditional animal farming, which has significant environmental impacts<sup>1</sup>. Fermentation presents a transformative solution for producing sustainable protein while addressing the environmental challenges posed by conventional protein sources<sup>2</sup>. Among the various substrates proposed for fermentation processes, marine biowaste has garnered attention.

Marine biowaste, which includes fish by-products, marine invertebrates, and macroalgae residues, is rich in proteins, essential nutrients, and carbohydrates, making it an ideal substrate for biomass fermentation<sup>3</sup>. Furthermore, although 88% of fishery products are used for human consumption, more than 35% of the catch is wasted annually<sup>1</sup>. Thus, fermentation not only enhances protein content but could also mitigate the overexploitation of marine stocks and reduces waste.

WP2 aims to characterize (Task 2.2) and select biomasses suitable for fermentation based on their carbohydrate and protein content, as well as other compounds that may influence the fermentation process. Once analyzed, initial fermentation trials will be conducted to preselect the 10 most promising marine biomasses, taking into account market analysis (Task 2.3) and consumer responses (Task 2.4).

### 4. MARINE BY-PRODUCTS OVERVIEW

#### 5.1 Types of Marine By-Products

Marine by-products are a valuable yet underutilized resource in the seafood industry, with potential applications in food, nutraceuticals, biomaterials, and animal feed. Derived from fish, mollusks, cephalopods, and macroalgae, these by-products are rich in proteins, lipids, minerals, and bioactive compounds, making them highly suitable for biotechnological valorization.

As part of WP2 of PROMISEANG, an initial screening was performed on over 30 distinct biomass samples and cooking waters, sourced from Jealsa, AlgaPlus, and IberAgar. These samples were categorized as follows: 14 fish by-products, such as: heads, bones, viscera, and skins. 3 mollusk samples from mussel, 3 cephalopod by-products, including skin, gladius and viscera, 6 macroalgae samples and 4 cooking waters from fish, mollusks, and cephalopods, classified as effluents from the canning industry. These samples were pre-selected by industry partners for further characterization and are detailed in *Annex I*. To preserve their integrity and enable future analyses, all samples underwent lyophilization. The complete protocols for sample logistics and analysis were previously reported in deliverable D2.1.

#### 5.2 Characteristics of Marine By-Products

The PROMISEANG project goes beyond protein extraction, emphasizing the valorization of marine biomass for recovering new alternative proteins and other high-value biomolecules. To support these goals, a detailed characterization of 30 samples was performed to assess their composition and suitability for biotechnological applications, including fermentation processes.

The characterization conducted in Task 2.2 confirmed that fish, mollusk, and cephalopod by-products are primarily rich in proteins and lipids, whereas macroalgae exhibit a higher carbohydrate content. However, the composition varied considerably depending on the type of sample. Protein content in fish by-products ranged widely, from 30% to 70%, while carbohydrate levels remained relatively low. Similarly, mollusk and cephalopod by-products displayed a broad spectrum of protein (5.04% to 70.02%) and carbohydrate (1.19% to 32.41%) concentrations. In contrast, macroalgae exhibited moderate protein levels but were predominantly carbohydrate-based (from 45.80% to 59.41%). Notably, carbon and nitrogen content analysis (**Table 1**) revealed that, while these samples contain significant protein levels, their C/N ratio suggests potential feasibility for

fermentation. By-products with lower C/N ratios (<10) may provide an adequate nitrogen source for microbial growth, while those with higher ratios may require supplementation to optimize fermentation efficiency.

Table 1. By-products classification according to the ratio Carbon:Nitrogen content.

| C/N Ratio |                                                                                                                                                                                                                                                       |
|-----------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| < 5       | Squid, tuna and octopus cooking broth, YF tuna tablet, SK tuna tails, YF tuna tails, mackerel head, squid gladius, squid skin, YF tuna viscera, tuna decanter meat, mussel broken viands, horse mackerel head, SK tuna viscera, SK tuna central spine |
| 5 - 7.5   | YF tuna skin, <i>Porphyra umbilicalis</i> , mussel epibiont, mussel cooking broth, <i>Ulva rigida</i> , YF tuna dorsal fin                                                                                                                            |
| 7.5 – 10  | <i>Gelidium corneum</i> , SK tuna heads                                                                                                                                                                                                               |
| > 10      | <i>Cyrtosena osneoides</i> , squid viscera, <i>Ruguloteryx akamurai</i> , <i>Saccharina latissima</i> , turbot viscera, mussel shell                                                                                                                  |

**Note.** Yellowfin (YF) and Skipjack (SK)

The analysis of the amino acid profile revealed a balanced composition of essential (EAA) and non-essential (n-EAA) amino acids across all marine by-products. While the amino acid profile itself does not directly impact the feasibility of fermentation, it is expected that bioconversion processes during single-cell protein production will modify this composition, potentially enhancing the digestibility and bioavailability of the resulting protein fractions <sup>4</sup>.

The lipids and mineral composition varied significantly between by-products. Some by-products contained notable levels of polyunsaturated fatty acids (PUFAs), particularly omega-3 compounds, which are recognized for their health benefits. These bioactive lipids hold significant potential for nutraceutical development, particularly in functional foods and dietary supplements <sup>5</sup>. Given their nutritional relevance, certain by-products may be better suited for alternative valorization routes, such as lipid extraction, rather than direct fermentation. Regarding mineral composition, some samples (particularly those from mollusks) were identified as rich sources of calcium and trace elements, which could be repurposed for biotechnological, agricultural, or biomaterial applications. Additionally, macroalgae-based samples were identified as promising sources of iodine and other micronutrients. High mineral content in specific by-products suggests potential use in biodegradable packaging, biomedical innovations, and pH regulation in fermentation processes.

Finally, several analyses were conducted to evaluate the safety of the selected by-products, assessments were conducted to evaluate heavy metal concentrations, and biotoxin levels. These factors are essential for determining the suitability of samples for fermentation, as excessive concentrations of certain compounds could inhibit microbial growth or pose health risks. These safety parameters also played a key role in selecting the final samples, and further details will be discussed in the next section.

## 5. SELECTION CRITERIA FOR MARINE BY-PRODUCTS

### 6.1 Criteria and considerations

Biomasses must supply essential nutrients, particularly carbon, nitrogen sources and minerals. Besides, the presence of inhibitory substances such as heavy metals or toxins should be addressed, due to directly impacting microbial metabolism and enzyme activity, ultimately affecting fermentation efficiency. Therefore, in selecting the biomasses, three primary criteria related to the essential requirements for microorganism

cultivation were considered, along with an additional factor addressing the operational and technical aspects of the project.

#### - Hazardous compounds.

The first criterion for selection was based on the content of heavy metals and/or biotoxins present in the samples, as these could pose a biological and/or food safety risk to animals or humans. Additionally, an excessive concentration of phenolic compounds or metals that could potentially inhibit microbial growth was also considered.

Analysis conducted in Task 2.2 by the AA1 and IBPRS groups did not reveal the presence of biotoxins or cytotoxic compounds. Regarding heavy metal content, the maximum permissible concentrations for lead, cadmium, mercury, and arsenic were established according to Codex Alimentarius standards <sup>7</sup>. All samples had concentrations below the established limits; however, *Cystoseira ustenoides* and Octopus Cooking Broth exhibited higher arsenic levels. According to Codex Alimentarius the maximum allowable limit for total arsenic is 0.5 mg/kg on a wet weight basis. Our results showed arsenic concentrations exceeding 100 mg/kg on a dry weight basis, thus in terms of wet biomass, the arsenic concentrations fell below the established limit. Nonetheless, due to the potential for arsenic accumulation in microbial biomass, which could pose risks in the final product, these biomasses were excluded from the fermentation process. Following this precautionary approach, *Saccharina latissima*, which had arsenic concentrations exceeding 50 mg/kg, was also excluded from the selection.

Initially, the total phenol content was also considered as an exclusion criterion, since several studies have indicated that concentrations exceeding 0.5 g/L can inhibit microbial growth. However, the Folin-Ciocalteu method, while widely used for the determination of phenolic compounds, is not recommended for samples with high soluble protein content, as it may lead to overestimation of the measurements. Therefore, this parameter was not considered.

#### - Protein content

The next criterion for the preselection of the 10 biomasses was protein content. In PROMISEANG, the fermentation process will be conducted with the objective of increasing the final protein content and enhancing its quality in terms of digestibility and amino acid profile. However, according to bibliographic references, the maximum yield of microbial protein or single-cell protein obtained through fermentation ranges from 30% to 70%, depending on the type of microorganism used <sup>8-10</sup>. Additionally, a nitrogen source is essential for microbial growth, and its requirement varies depending on the microorganism. Consequently, the final protein content may be lower than that of the raw biomass but with different characteristics. Thus, biomasses have been classified into three categories: low-protein content (<20%), medium-protein content, and high-protein content (>60%).

Table 2. Protein content of each by-product expressed as a percentage of dried biomass.

| Protein content (%) |                                                                                                                                                                                                                                                   |
|---------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| < 20                | Mussel Shell, <i>Ulva rigida</i> , <i>Cyrtosena osneoides</i> , <i>Saccharima latissima</i> <i>Ruguloteryx akamurai</i> , Mussel Cooking Broth, <i>Gelidium corneum</i> , Mussel epibiont                                                         |
| 30 - 60             | <i>Porphyra umbilicalis</i> , Turbot Viscera, YF tuna Dorsal Fin, SK tuna Tails, SK tuna Heads, YF tuna Tails, YF tuna Skin, Tuna Cooking Broth, Squid Viscera, Tuna Decanter meat, Mussel Broken Viands and Breeds, Squid Skin, SK tuna Skin, SK |

tuna Central Spine, Octopus Cooking Broth

> 60

Horse mackerel Head, Squid Gladius, Mackerel Head, SK tuna Viscera, Squid Cooking Broth, YF tuna Tablet, YF tuna Viscera

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**Note.** Yellowfin (YF) and Skipjack (SK)

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Biomasses from the first two levels will be preferably selected, as according to bibliographic references, the final biomass content will not exceed 70%. Consequently, fermentative technology would not be suitable for their recovery and valorization, making another type of procedure more appropriate. However, in order to corroborate this hypothesis, at least one biomass will be selected from the highest level.

#### - **Carbohydrate content**

Besides the nitrogen source, microorganisms require a carbon supply for their proper growth and development. Typically, carbon is present in a proportion at least 10 times greater than the nitrogen source. Therefore, it will be necessary to select biomasses that provide monomeric sugars, polyols, or polysaccharides that can be metabolized by the microorganisms. In this sense, Mussel cooking broth and algae exhibited carbohydrate contents of 32% and 55%, respectively, making both excellent sources of carbon.

#### - **Biomass availability and variability.**

Besides the fact that each microorganism has distinct nutritional needs, it is also essential to consider the availability and the variability inherent in fishery and canning by-products<sup>3,6</sup>. PROMISEANG aims to introduce new products to the market, and as such, it must ensure a continuous supply throughout the entire year. Consequently, it will be crucial to consider the volumes produced by the companies for each by-product and the closed fishing seasons. Besides the natural variability between individuals, factors such as climate, water conditions, catch area, season, or even the year can influence the composition of the byproduct. Therefore, biomasses with similar compositions will not be differentiated by species, but by the type of by-product generated. An example of this is the skin of the tuna species Yellowfin and Skipjack, where the protein content ranges from 40 to 56 %, and the carbohydrate content ranges from 15 to 12 %, respectively. This biomass will be considered as tuna skin and used interchangeably, thus aiming to reduce the long-term variability effects and increase the final volumes of the by-product.

### 6.2 Key Properties for Successful Fermentation

PROMISEANG is an ambitious project focused on the production of single-cell protein using bacteria, yeast, and fungi. Each type of microorganism requires specific environmental conditions (pH, temperature, oxygen levels) and nutritional factors (carbon, nitrogen, minerals) for optimal growth, all of which must be carefully controlled to maximize fermentation performance and product yield. Additionally, the type of fermentation (whether submerged or solid-state) also influences the process. This implies that each culture should be optimized based on each type of biomass and microorganism used. However, one of the main challenges in developing biological treatments for organic biomasses is the high degree of variability between samples. Therefore, the aim of this project is to develop versatile fermentative processes, ensuring that biomass selection is based on a defined range of characteristics rather than specific values to optimize the growth of each microorganism.

### 6.3 Selected Marine By-Products for Biomass Fermentation

From the application of the four established criteria, 10 by-products were selected. As shown in Table 3, mussels and tuna were the primary sources of by-products. According to Jealsa data, between 80,000 and

100,000 tons of mussel shells are generated annually in Galicia. Mussel epibiota includes all the biomass that grows on the surface and is cleaned before sale. Although there are no specific production figures, according to the volume of mussel shells produced it is expected that this by-product was produced in large quantities. Broken viands and breeds account for a total of 75 tons per year, while mussel cooking broths reach 10,000 tons annually. Regarding the most representative fish species, tuna stands out, generating 635 tons of central spine, approximately 1,000 tons of skins, and another 1,000 tons of tails per year, along with 1,500 tons of viscera. Of these volumes, approximately 80% corresponds to the Yellowfin species and 20% to Skipjack. As for tuna cooking broths, up to 21,150 tons can be produced annually. The quantities produced annually could be sufficient for the development of innovative biotechnological processes that coexist symbiotically with the fishing and canning industries while simultaneously minimizing an environmental problem.

Table 3. Selected Marine By-Products for Biomass Fermentation

| Mollusk                         | Fish               | Algae                                       |
|---------------------------------|--------------------|---------------------------------------------|
| Mussel Epibiota                 | Tuna Tails         | <i>Ulva rigida</i>                          |
| Mussel Cooking Broth            | Tuna Viscera       | Industrial waste of <i>Gelidium corneum</i> |
| Mussel Broken Viands and Breeds | Tuna Skin          |                                             |
|                                 | Tuna Central Spine |                                             |
|                                 | Tuna Cooking Broth |                                             |

All selected by-products have been shown to be safe for use as fermentation substrates, as well as for human and environmental health. Additionally, they are representative of the three protein levels shown in Table 1. Furthermore, the criterion for carbon source content is met by including the two algae samples and mussel cooking broth. Thus, this preselection theoretically covers all the nutritional ranges that microorganisms may require for protein production. To validate this hypothesis, the next step involved conducting solid-state and liquid fermentation processes using the selected by-products.

## 6.4 Market analysis

### 6.4.1 Growth and Market Size of Alternative Proteins

The global alternative protein market has experienced **significant growth**, with an estimated value of **\$15–16.8 billion in 2022** and a projected **compound annual growth rate (CAGR) of 9.84% to 15.6%** over the next 7 to 13 years. By **2035, the market is expected to reach approximately \$290 billion**, with alternative proteins accounting for **11% of the total protein market**.

This growth is driven by:

- **Sustainability concerns**, as traditional livestock production contributes 14.5% of anthropogenic greenhouse gas emissions.
- **Shifting consumer preferences**, with more than **40% of consumers in key markets** (EU, U.S., and Asia) actively seeking sustainable protein sources.
- **Government support**, particularly in the **EU Green Deal and Horizon Europe programs**, which encourage circular bioeconomy initiatives.

#### 6.4.2 Market Prospects and Opportunities

##### - Food applications

- The global alternative protein market for human consumption is expected to grow significantly, driven by increasing consumer demand for sustainable, nutritious, and ethically sourced protein options.
- **High-protein food applications, including meat and dairy alternatives, bakery products, and plant-based seafood, are gaining traction.** These products offer a healthier and more sustainable alternative to animal-based proteins, making them popular among health-conscious and environmentally aware consumers.
- Marine-derived proteins, such as fish proteins, algae, and krill, are increasingly used in sports nutrition, protein-enriched snacks, and fortified beverages. These proteins are recognized for their high digestibility and bioactive properties, such as omega-3 fatty acids and antioxidants, which support overall health and muscle recovery.
- Functional ingredients derived from marine biomass, including **omega-3 fatty acids, collagen, and bioactive peptides**, are being incorporated into dietary supplements and nutraceuticals. These ingredients are particularly popular for their anti-inflammatory, joint-supporting, and heart-health benefits.
- The growing trend of clean-label and natural ingredients has significantly boosted interest in marine-based proteins as a sustainable and minimally processed alternative. Consumers are increasingly seeking transparent and natural food labels, prompting food manufacturers to adopt marine-derived ingredients for their nutritional value and sustainability.
- Biomass fermentation and biotechnology are playing a key role in the production of alternative proteins, allowing the extraction of high-value functional ingredients from sources such as microalgae and marine byproducts.

##### - Animal Feed (Aquaculture, Livestock, and Pet Food)

- The global alternative protein market for animal feed is expected to grow substantially, driven by the increasing demand for sustainable and high-quality protein sources that minimize the environmental impact of livestock and aquaculture farming.
- The global alternative protein market for animal feed is projected to grow from **\$3.96 billion in 2023 to \$8.2 billion by 2033**, with a **CAGR of 7.5%**. This growth is driven by the rising incidence of **cattle diseases, epidemics, and the demand for nutritional alternatives** in animal diets.
- The **aquaculture feed market alone is projected to reach \$80 billion by 2030**, as the industry seeks **alternative protein sources** to replace **fishmeal and soybean meals**, which have high environmental footprints. Plant-based, insect, and marine-derived proteins are gaining ground in replacing traditional feed ingredients to promote sustainability in aquaculture.
- The demand for alternative proteins in pet food is rising due to growing pet ownership and consumer interest in sustainable, high-protein diets. The European pet food market alone is valued at €55.02 billion and is expected to grow at a CAGR of 7.47% from 2024 to 2029.
- Pet food manufacturers are shifting toward **sustainable and functional protein sources**, favoring **marine-based ingredients**. These proteins are favoured for their high digestibility, bioactive compounds, and positive impact on pet health, including skin, coat, and digestion.
- The **valorisation of marine byproducts** (such as **fish heads, bones, and viscera**) into **high-protein feed ingredients** aligns with **EU sustainability policies**, reducing **waste and promoting resource efficiency**.

## - Non-Food Applications (Pharmaceuticals, Nutraceuticals, and Cosmetics)

- The global nutraceutical market is projected to reach **\$626.02 billion by 2029, growing at a CAGR of 5.09%**. This growth is fueled by increasing consumer demand for functional foods, dietary supplements, and natural health solutions. Marine-derived proteins such as omega-3 fatty acids, collagen, and bioactive peptides are widely used in heart health, anti-aging, and immune-boosting products.
- The marine-based cosmetic ingredients market is expanding, driven by sustainable beauty trends and the increasing demand for natural bioactive ingredients. Marine-derived collagen, algae, and seaweed extracts are used in skincare products for their anti-aging, hydrating, and soothing properties.
- The global protein therapeutics market is expected to double **from \$283.64 billion in 2020 to \$566.66 billion by 2030, at a CAGR of 7.1%**. Proteins such as chitosan, marine collagen, and peptides are being used for drug delivery systems, wound healing, and tissue engineering.
- The encapsulation market, used in drug delivery and food applications, was valued at \$35 billion in 2021, with a CAGR of 12.5% (2021-2028). Hydrogels, derived from marine proteins and polysaccharides, are gaining traction in biomedical and cosmetic formulations.
- Marine waste valorization, the process of converting marine byproducts into valuable products, aligns with EU policies on the circular bioeconomy. This process is opening new regulatory and funding opportunities to promote the sustainable use of marine resources and support **the development of new bio-based industries**.

### 6.5 Inclusion of the food system actors' views for selection of biomass samples

The protocol hereby described would undoubtedly benefit of the view of the different actors involved in the food system, as a complementary information to those collected in the market analysis. However, and due to the amendment of the original Grant Agreement of the Promiseang project, the task corresponding to collection of the actors' view and inputs has been extended till June 2025, making impossible for the Promiseang partners to gather all required information at the time of publishing this deliverable (February 2025).

PROMISEANG is highly committed to excellence on results and offering results which are both easily understandable by any general audience without affecting to its scientific validity, as innovative outcomes that help boosting research and exploitation of the uncovered knowledge.

Therefore, the information pertaining to this section will be available in D2.4 - Factors blocking the sustainability of alternative protein systems in EU, which will be released on June 2025.

### 6.6 Justification of selected biomass samples for feed and non-food applications

The selection of **10 biomass samples** was strategically aligned with **market opportunities in food, feed and non-food applications** based on **biochemical composition, industrial relevance, and sustainability factors**.

| Selected Biomass       | Food Potential                                              | Feed Potential                                  | Non-Food Potential                                         |
|------------------------|-------------------------------------------------------------|-------------------------------------------------|------------------------------------------------------------|
| <b>Mussel Epibiota</b> | Potential inclusion in high-protein snacks and supplements. | Prebiotic effects, mineral source for aquafeed. | Rich in bioactive compounds, potential for nutraceuticals. |

|                                                    |                                                                                                                            |                                                                                                                                                     |                                                                                                                   |
|----------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------|
| <b>Mussel Cooking Broth</b>                        | Functional ingredient in soups, broths, and protein beverages. Natural extract for canned and frozen products.             | High in essential amino acid that supports fish growth and selenium and taurine for pet food complete nutritional formulations & palatants & baits. | Antioxidant-rich extracts for pharma & cosmetics & nutraceuticals.                                                |
| <b>Mussel Viands Breeds</b>                        | Marine protein and carbohydrates source for plant-based meat substitutes.                                                  | Source of marine protein for livestock and aquaculture feed & pet food & palatants & baits.                                                         | Valorisation into marine collagen & peptides.                                                                     |
| <b>Tuna Tails</b>                                  | Source of protein hydrolysates for sports nutrition.                                                                       | Sustainable fishmeal alternative.<br>Source of hydrolysed protein for pet food and aquaculture.                                                     | Omega-3 extraction for nutraceuticals. Source of collagen for nutraceuticals. Source of nitrogen for fertilizers. |
| <b>Tuna Viscera</b>                                | Protein concentrates for fortified food applications.                                                                      | High in lipids (EPA/DHA) for fish & pet food.                                                                                                       | Rich in enzymes & proteins, applicable in pharma. Source of nitrogen for fertilizers.                             |
| <b>Tuna Skin</b>                                   | Source of marine collagen for protein-enriched foods. Source of gelatine as a gelling agent in food, pharma and cosmetics. | Collagen-rich, used in pet food. Gelatine used in aquaculture and for baits formulation.                                                            | Source of marine collagen for cosmetics. Source of collagen for nutraceuticals.                                   |
| <b>Tuna Central Spine</b>                          | Calcium-enriched ingredient for plant-based dairy alternatives                                                             | High mineral content, useful in feed fortification, pet food bone recovering.                                                                       | Calcium & phosphorus source for supplements. For dental & bone regeneration applications.                         |
| <b>Tuna Cooking Water</b>                          | Functional peptides for food preservation and formulation as a natural extract flavour.                                    | High protein hydrolysates for aquafeed & petfood & bait formulations.                                                                               | Functional peptides for pharma & nutraceutical applications. Source of nitrogen for fertilizers.                  |
| <b>Industrial Waste of <i>Gelidium corneum</i></b> | Food additive in functional foods and dietary fibres.                                                                      | Rich in prebiotic polysaccharides, gut health booster.                                                                                              | Agar extraction for cosmetics & pharmaceuticals.                                                                  |

|                    |                                                             |                                                                          |                                                    |
|--------------------|-------------------------------------------------------------|--------------------------------------------------------------------------|----------------------------------------------------|
| <i>Ulva rigida</i> | Source of bioactive polysaccharides for food fortification. | High carbohydrate & protein content, optimal for microbial fermentation. | Source of bioactive polysaccharides for cosmetics. |
|--------------------|-------------------------------------------------------------|--------------------------------------------------------------------------|----------------------------------------------------|

## 6. SCREENING OF BIOMASS AS SUBSTRATE FOR SOLID STATE AND SUBMERGED FERMENTATION

### 7.1 Solid-State Fermentation

As previously stated, each type of microorganism and culture employed has specific requirements. Solid-state fermentation (SSF) is characterized by the use of substrates with little to no free-flowing water. Therefore, solid samples were used as main substrate, while broths will be utilized in subsequent cultures as moisturizers, or as supplements to provide more nutrients for the fermentation process. Another particular characteristic of SSF is that the final biomass will be the protein-rich product intended for the development of new foods and/or animal feed. For this reason, *Gelidium corneum* waste was not considered for SSF, as it comes from an industrial process in which bentonite is used for the extraction of agar, and this mineral, which is not suitable for human consumption, could be present in the final composition.

Thus, the initial trials were done only with Mussel Epibiota, Mussel Broken Viands and Breeds, Tuna Tails, Tuna Viscera, Tuna Skin, Tuna Central Spine and *Ulva rigida*. For this, 5 g of each biomass were dispensed into 250 mL Erlenmeyer flasks. The samples were moistened with distilled water and sterilized in an autoclave for 15 minutes at 121 °C. Once the temperature reached room temperature, the substrates were inoculated with two different fungal strains: *Aspergillus oryzae* and *Rhizopus oligosporus*. Both fungal strains were obtained from the Spanish Type Culture Collection (CECT), specifically CECT 2094 and CECT 20169. The cultures were incubated for 10 days at 30 °C, and daily observations were made to monitor the presence or absence of growth.

Except for the skin, all the biomasses allowed the growth of both types of fungi. **Figure 1** shows the type of growth and development of the fungi on several of the biomasses, validating the potential of marine by-products for the cultivation of these two species.

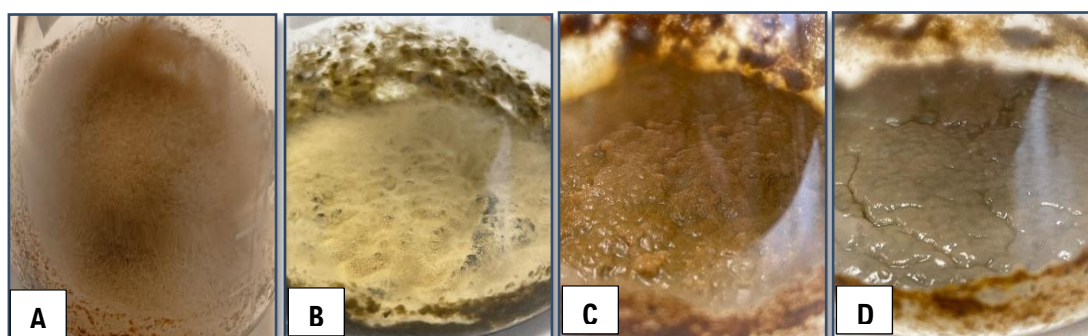


**Figure 1.** Growth of: A) *Rhizopus oligosporus* on central spine; B) *Aspergillus oryzae* on *U. rigida*; C) *Aspergillus oryzae* on tuna viscera; D) *Aspergillus oryzae* on Mussel Broken Viands and Breeds.

Preliminary results indicate that *R. oligosporus* increases the final protein content in mussel epibiota, Mussel Broken Viands and Breeds, and *Ulva rigida*. However, *Ulva* will be discarded as a primary substrate due to its final protein yield being below 15%, whereas the other biomasses exceed 50%. Additionally, notable mycelium development is observed when the central spine is used as a substrate. Therefore, for the optimization processes, mussel epibiota, Mussel Broken Viands and Breeds, and central spine will be used in combination

with substrate rich in carbon source to enhance the final protein content.

Regarding the cultures with *A. oryzae*, the results showed that the fungus significantly reduced the final protein content in the central spine and viscera. Similar to *Rhizopus*, the protein content in *Ulva* was too low for it to be used as a primary nutrient source. However, it will be used as a carbon source supplement for mussel epibiota and Mussel Broken Viands and Breeds by-products to enhance the final protein content.

## 7.2 Submerged Fermentation

With the byproducts list as starting point, for submerged fermentation, the potential to generate liquid culture media that meet the necessary requirements for microbial growth was evaluated. The main requirement is a high carbohydrate content (carbon source). The simpler the carbohydrates, the better the microbial uptake and metabolism, with glucose (monosaccharide) being superior to sucrose (disaccharide) and starch (polysaccharide). On the other hand, the contribution of a nitrogen source is essential. This nitrogen source is usually provided by an exogenous addition of salts and/or yeast extract, which has a high content of free amino nitrogen, alpha-amino acids, and small peptides.

Based on this, both *Ulva rigida* and *Gelidium corneum* were initially selected. The exact composition of both algae was input into our artificial intelligence platform *Albatros*, which returned a comprehensive analysis of the microorganisms belonging to MOA Foodtech's proprietary collection for the fermentation of the selected byproducts. Table 4 shows the requirements of each of these microorganisms and the expected biomass yield for each one. This simulation allowed for the validation of the selection of MOA-003, MOA-016, and MOA-223 as the yeasts (003 and 016) and bacteria (223) to begin the growth trials and optimization of the small-scale fermentation process before proceeding to the bioreactor phase.

Table 4. Selection, expected requirements and yield prediction in submerged fermentation of algae byproducts

| MOA collection |            | Nitrogen source                                      |                                                                                    | Max. biomass yield (g/L) |                   |
|----------------|------------|------------------------------------------------------|------------------------------------------------------------------------------------|--------------------------|-------------------|
| Microorganism  | MOA strain | <i>U. rigida</i>                                     | <i>G. corneum</i>                                                                  | <i>U. rigida</i>         | <i>G. corneum</i> |
| Bacteria       | MOA-233    | 30mM K <sub>2</sub> HPO <sub>4</sub>                 | 45mM K <sub>2</sub> HPO <sub>4</sub>                                               | 15.08                    | 37,53             |
| Yeast          | MOA-016    | 28mM (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> | 0.2M (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub><br>15.6 mM NaHPO <sub>4</sub> | 11.03                    | 42,97             |
| Yeast          | MOA-003    | 1mM K <sub>2</sub> HPO <sub>4</sub>                  | 6mM NH <sub>4</sub> OH<br>27mM K <sub>2</sub> HPO <sub>4</sub>                     | 10.27                    | 30,81             |

Both byproducts of algal origin were treated through acid hydrolysis at the University of Lisbon and began to formulate culture media for initial liquid fermentation tests. Since the carbon to nitrogen ratio requirements of the selected microorganisms range between 20:1 and 30:1, and both *Ulva rigida* and *Gelidium corneum* have or even exceed this ratio, the prediction establishes that the exogenous contribution of nitrogen source is small and could be provided by nitrogen salts. In this regard, the viscera, skin, and central spine of tuna fish—regardless of species and depending on the timing of the catches by the fishing companies—are, in our opinion, good candidates to replace the exogenously added salt/yeast extract. As soon as we get access to these byproducts, we aim to substitute the salt/yeast extract with them.

Regarding the issue of heavy metal presence, we need to generate a sufficient amount of final product to conduct thorough analyses of these metals. For this purpose, we need to complete the fermentation optimization process that we are carrying out. Currently, we are in the phase of optimizing growth conditions prior to scaling up to bioreactor trials, so these analyses have been postponed. While the literature suggests that this should not pose a problem<sup>11,12</sup>, we have strategies and measures that can mitigate this issue, starting with increasing the number of washes of the final biomass harvested and followed using various filtration-

concentration methods.

## 7. CONCLUSION

The four criteria established (Hazardous compounds, protein content, carbohydrate content, biomass availability and variability) for the preselection of marine biomasses enabled a successful identification of suitable substrates for fermentation. All selected biomasses demonstrated potential for fermentation processes. Specifically, Mussel Epibiota, Mussel Broken Viands and Breeds, and Central Spine were suitable for solid-state fermentation, while Skin, Viscera, *Ulva rigida*, and *Gelidium corneum* were better suited for submerged fermentation as primary substrates.

The next phase will focus on optimization, involving biomass combinations and an assessment of abiotic parameters. Furthermore, the selection of marine biomass in PROMISEANG aligns with key market trends, ensuring:

- **Expansion into Food and Non-Food Markets:** Leveraging collagen, omega-3s, and marine polysaccharides for food, nutraceutical, and cosmetic applications.
- **Sustainable Feed Innovation:** Addressing protein shortages in aquafeed and pet food through the utilization of marine by-products.
- **Regulatory Compliance and Market Readiness:** Supporting EU sustainability goals while responding to consumer demand for marine-derived bioactive compounds.
- **Innovation in Fermentation Technologies:** Advancements in biomass fermentation processes enhance protein conversion rates, improving commercial viability.

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## 9. ANNEXES

**Annex 1.** List of characterized by-products according to the protocols of deliverable D2.1.

| Nº | By-product                     | Specie                                                                                               | Type of substrate | Origin          | Provider |
|----|--------------------------------|------------------------------------------------------------------------------------------------------|-------------------|-----------------|----------|
| 1  | Heads (STH)                    | <b>Skipjack Tuna</b> ( <i>K. pelamis</i> )                                                           | Fish              | Atlantic Ocean  | Jealsa   |
| 2  | Tails (STT)                    | <b>Skipjack Tuna</b> ( <i>K. pelamis</i> )                                                           | Fish              | Atlantic Ocean  | Jealsa   |
| 3  | Central Spine (STCS)           | <b>Skipjack Tuna</b> ( <i>K. pelamis</i> )                                                           | Fish              | Atlantic Ocean  | Jealsa   |
| 4  | Viscera (STV)                  | <b>Skipjack Tuna</b> ( <i>K. pelamis</i> )                                                           | Fish              | Atlantic Ocean  | Jealsa   |
| 5  | Skins (STS)                    | <b>Skipjack Tuna</b> ( <i>K. pelamis</i> )                                                           | Fish              | Atlantic Ocean  | Jealsa   |
| 6  | Tablet (YTTB)                  | <b>Yellowfin Tuna</b> ( <i>T. albacares</i> )                                                        | Fish              | Atlantic Ocean  | Jealsa   |
| 7  | Dorsal Fin (YTDF)              | <b>Yellowfin Tuna</b> ( <i>T. albacares</i> )                                                        | Fish              | Atlantic Ocean  | Jealsa   |
| 8  | Tails (YTT)                    | <b>Yellowfin Tuna</b> ( <i>T. albacares</i> )                                                        | Fish              | Atlantic Ocean  | Jealsa   |
| 9  | Viscera (YTV)                  | <b>Yellowfin Tuna</b> ( <i>T. albacares</i> )                                                        | Fish              | Atlantic Ocean  | Jealsa   |
| 10 | Skin (YTS)                     | <b>Yellowfin Tuna</b> ( <i>T. albacares</i> )                                                        | Fish              | Atlantic Ocean  | Jealsa   |
| 11 | Decanter Meat (TD)             | <b>Tuna</b> ( <i>K. pelamis</i> and <i>T. albacore</i> )                                             | Fish              | Atlantic Ocean  | Jealsa   |
| 12 | Cooking Broth (TB)             | <b>Tuna</b> ( <i>K. pelamis</i> and <i>T. albacore</i> )                                             | Fish              | Atlantic Ocean  | Jealsa   |
| 13 | Viscera (TV)                   | <b>Turbot</b> ( <i>S. maxima</i> )                                                                   | Fish              | Spain           | Jealsa   |
| 14 | Head (HMH)                     | <b>Horse mackerel</b> ( <i>T. trachurus</i> )                                                        | Fish              | Spain           | Jealsa   |
| 15 | Head (MH)                      | <b>Mackerel</b> ( <i>M. poutassou</i> )                                                              | Fish              | Spain           | Jealsa   |
| 16 | Epibiota (ME)                  | <b>Mussel</b> ( <i>M. galloprovincialis</i> )                                                        | Mollusk           | Galicia (Spain) | Jealsa   |
| 17 | Broken Viands and Breeds (MBV) | <b>Mussel</b> ( <i>M. galloprovincialis</i> )                                                        | Mollusk           | Galicia (Spain) | Jealsa   |
| 18 | Shell (MS)                     | <b>Mussel</b> ( <i>M. galloprovincialis</i> )                                                        | Mollusk           | Galicia (Spain) | Jealsa   |
| 19 | Cooking Broth (MB)             | <b>Mussel</b> ( <i>M. galloprovincialis</i> )                                                        | Mollusk           | Galicia (Spain) | Jealsa   |
| 20 | Cooking Broth (OB)             | <b>Octopus</b> ( <i>O. vulgaris</i> )                                                                | Cephalopod        | Morocco         | Jealsa   |
| 21 | Cooking Broth (SB)             | <b>Squid</b> ( <i>S. officinalis</i> , <i>D. gigas</i> , <i>I. argentinus</i> , <i>L. vulgaris</i> ) | Cephalopod        | Galicia (Spain) | Jealsa   |
| 22 | Gladius (SG)                   | <b>Squid</b> ( <i>S. officinalis</i> , <i>D. gigas</i> , <i>I. argentinus</i> , <i>L. vulgaris</i> ) | Cephalopod        | Galicia (Spain) | Jealsa   |

|    |                  |                                                                                                      |            |                 |          |
|----|------------------|------------------------------------------------------------------------------------------------------|------------|-----------------|----------|
| 23 | Skin (SS)        | <b>Squid</b> ( <i>S. officinalis</i> , <i>D. gigas</i> , <i>I. argentinus</i> , <i>L. vulgaris</i> ) | Cephalopod | Galicia (Spain) | Jealsa   |
| 24 | Viscera (SV)     | <b>Squid</b> ( <i>S. officinalis</i> , <i>D. gigas</i> , <i>I. argentinus</i> , <i>L. vulgaris</i> ) | Cephalopod | Galicia (Spain) | Jealsa   |
| 25 |                  | <i>Ulva rigida</i>                                                                                   | Algae      | Portugal        | AlgaPlus |
| 26 |                  | <i>Saccharima latissima</i>                                                                          | Algae      | Portugal        | AlgaPlus |
| 27 |                  | <i>Porphyra umbilicalis</i>                                                                          | Algae      | Portugal        | AlgaPlus |
| 28 | Industrial waste | <i>Gelidium corneum</i>                                                                              | Algae      | Portugal        | IberAgar |
| 29 |                  | <i>Cyrtosena usneoides</i>                                                                           | Algae      | Portugal        | AlgaPlus |
| 30 |                  | <i>Ruguloteryx akamurai</i>                                                                          | Algae      | Portugal        | AlgaPlus |