

INNOAQUA PROJECT

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D4.1: Initial report on product prototypes specifications

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List of Acronyms

Abbreviation / Acronym	Description
AA's	Amino acids
DHA	Docosahexaenoic Acid
DW	Dried Weight
CO₂ eq.	Carbon Dioxide Equivalent
EC	European Commission
EPA	Eicosapentaenoic Acid
EPD	Environmental Product Declaration
EU	European Union
FPH's	Fish Protein Hydrolysates
HDPE	High Density Polyethylene
IMTA	Integrated Multitrophic Aquaculture
KPI	Key Performance Indicator
LCA	Life Cycle Assessment
LCC	Life Cycle Cost Assessment
LDPE	Low Density Polyethylene
M	Month
PE	Polyethylene
PP	Polypropylene
ppm	Parts Per Million
PUFA's	Polyunsaturated Fatty Acids
RAS	Recirculating Aquaculture Systems
SLCA	Social Life Cycle Assessment
T	Task
WP	Work Package



Executive Summary

This document is part of the Task 4.1 of the INNOAQUA project and describes the different ingredients and product prototypes that will be developed in Work Package 4. The general description of each product and ingredient is provided, as well as specific information regarding its characteristics and regulatory, sustainability and consumer acceptance aspects that needs to be considered for the production and commercialization. Information regarding the expected quantities to be produced for each product and ingredient is also stated, and it is used to calculate the amount of biomass that will be necessary to produce in order to be able to obtain the desired amounts of ingredients and products.

Overall, this document aims to perform as a guideline for all biomass, ingredients, seafood products and packaging solutions producers along the whole INNOAQUA project, and it is a living document which will be subsequently updated whenever new knowledge about the products is obtained, or new technical decisions are made. The updates to this document will be included in the deliverables D4.5 (M30) and D4.6 (M48).



1. Introduction

The INNOAQUA project - Innovative approaches for an integrated use of algae in sustainable aquaculture practices and high-value food applications - aims to pave the path towards the upcoming sustainable and diversified EU land-based aquaculture industry by leaning on the demonstration and mainstreaming of innovative algae-based foods and solutions, using ecology, circularity, and digitalization approaches.

In a scenario where global food systems are being challenged due to the expected population growth, together with resource impoverishment and other environmental constraints, seafood has been identified as a vital source of food and a key component of a healthy diet. Nonetheless, decades of unsustainable overfishing practices are depleting aquatic ecosystems at a time when nearly one-fifth of all animal protein consumed by humans comes from seafood (1), reason why aquaculture has gained traction over wild fisheries. To ensure the future viability of the aquaculture sector and to unlock its potential to provide food with a lower carbon footprint (as stated in the Farm to Fork (F2F) Strategy of the European Green Deal), it is imperative to improve current technologies and management strategies, incorporating circular economy principles, optimising resources, reducing the operational costs, and minimising the environmental footprint. In this sense, algae (both microalgae and seaweed) have much potential, both for improving the sustainability of the production processes and as a direct food source to increase the seafood offer to consumers.

Within this context, INNOAQUA proposes an ambitious and efficient R&I workplan to develop and mainstream several solutions for the aquaculture industry involving the use of algae. Relying on a multidisciplinary consortium of renowned research centres, associations and companies with high industrial presence (11 companies) it is:

- (i) Demonstrating the feasibility and benefits of multi-trophic in-land cultivation management practices (i.e., RAS and IMTA) enhanced using the latest digital technologies.
- (ii) Contributing to the improvement of the sustainability and competitiveness of already-established value chains through the implementation of circular economy principles to minimise waste production in cultivation and processing facilities.



- (iii) Extracting high-added value ingredients from algae biomass and fish by-products to be used in the formulation of innovative seafood products, focusing from the beginning on social innovation approaches aimed at improving their societal acceptance and market penetration.

All this, enhanced by a robust outreaching strategy aimed at fostering knowledge transfer through an active engagement of relevant European and international actors, ultimately helping to maximize the project's scope and impact.

1.1. Purpose of the document

The purpose of INNOAQUA's Work Package 4 is to develop innovative seafood products by:

- (i) developing new ingredients based on the extracts obtained in WP3 from microalgae, macroalgae and fish-waste biomass,
- (ii) formulating these ingredients into nutritious seafood products and packaging solutions,
- (iii) performing tests to validate the performance, compared against existing solutions, to ensure consumer acceptance and to demonstrate safety, quality, and regulatory compliance,
- (iv) testing the stability and determine the shelf-life of ingredients and products.

In this context, Task 4.1, which started at M1 and will last until the end of the project, aims to define the critical specifications to be considered for each product prototype, including:

- i) a general description of the product,
- ii) compilation of regulatory aspects to be considered during manufacturing, labelling and commercialisation (including novel food approval),
- iii) essential attributes to be met during manufacturing, packaging and product use to ensure safety, quality, costs, sustainability, nutrition and consumer acceptance,
- iv) quantities to be produced and specific amounts of extracts required,
- v) methods and criteria for assessing performance, including benchmark comparisons with existing commercial products aims to define the specifications that must be



considered for the development and production of each seafood product prototype and packaging solution.

Thus, prior to the development of the other WP4 tasks, all critical specifications have been discussed and defined together with all the WP4 partners, as well as regulation, sustainability, and consumer acceptance experts within the INNOAQUA project. The present document summarizes all this information for each of the ingredients, seafood products and packaging solutions to be developed at T4.2, T4.3 and T4.4, respectively. For each of them, a summary of the compiled information will be provided below, following the i-v) points described just above. Due to the very incipient product design, for some of the specifications it is yet early to have very defined characteristics and requirements, and thus, it will be necessary to further develop them in the future. Some examples are the regulatory, sustainability and consumer acceptance attributes, which will further be studied and expanded during the development of WP5 and WP1, respectively. Another example is the definition of the process costs, which will strongly depend on the final product formulations and ingredients extraction protocols, which will be developed as part of WP4 and WP3, respectively. For all of them, a first approximation has been included in the present document but will later need to be reviewed and updated. The new information will be included in the further updates of the present document, in the deliverables D4.5 (M30) and D4.6 (M48).

Last but not least, this document also aims to serve as a first approach to the calculation of the quantities of each ingredient and product that will be produced within the INNOAQUA project, in order to assess that a sufficient amount of biomass is being produced in WP2.

1.2. Structure of the document

This document is divided into six sections:

Section 1 serves as an introduction.

Section 2 summarises the methodology that has been employed to compile all the required information.



Section 3 introduces the list of all the ingredients and products to be developed within the INNOAQUA project.

Section 4 presents the detailed description and specifications of each of the final seafood products and packaging solutions.

Section 5 provides the detailed information of the different ingredients, including the characteristics and requirements of each of them. The biomass characteristics is also introduced here.

Finally, **Section 6** presents some general conclusions of the deliverable and sets the next steps.

1.3. Relation to other project deliverables

As mentioned above, the present document summarizes information and inputs not only from all the WP4 partners, but also from the consumer experts from WP1, the biomass producers from WP2, the ingredient developers from WP3 and the regulatory and sustainability experts from WP5. Thus, T4.1 is an overarching task centralising inputs from across a wide range of project activities to ensure successful product formulations by ALGEMY, PESCANOVA, VIVA MARIS, LEITAT and ERANOVA.

The D4.1 report on product prototypes specifications will be subjected to permanent updates throughout the course of the INNOAQUA project in a structured feedback loop established between WP4, WP1 (NORCE) and WP5 (SUSTAINN and PERSEUS BVBA). This feedback loop will be reflected on the updates reported on D4.5 (M30) and D4.6 (M48), and will include the input provided by WP1 (NORCE) regarding consumer acceptance (D1.1, D1.2 and D1.3) and WP5 (SUSTAINN and PERSEUS BVBA) regarding sustainability (D5.1, D5.2, D5.5, D5.6, D5.7 and D5.8) and regulatory aspects (D5.3, D5.4 and D5.9). WP2 (NORCE and A4F) will also provide feedback regarding the biomass production (Task 2.4; Milestone 4), and WP3 (ALGEMY, A4F and LEITAT), regarding the processing of the biomass to extract the different ingredients (Tasks 3.2, 3.3 and 3.4 and Deliverables D3.2, D3.3 and D3.4).

Thus, a lot of feedback is expected along the development process, and especially once the production of ingredients (T4.2), final seafood products (T4.3) and packaging solutions (T4.4)

start. The current report (D4.1) will be updated at M30 (D4.5), coinciding with the finalization of the laboratory scale tests for the extraction of ingredients from tasks T3.2, T3.3 and T3.4 (WP3) and the kitchen-scale test for the final seafood products. Later, a final update will take part at the end of the project (M48, D4.6)), including the feedback from the industrial-scale production of both the ingredients and final products.

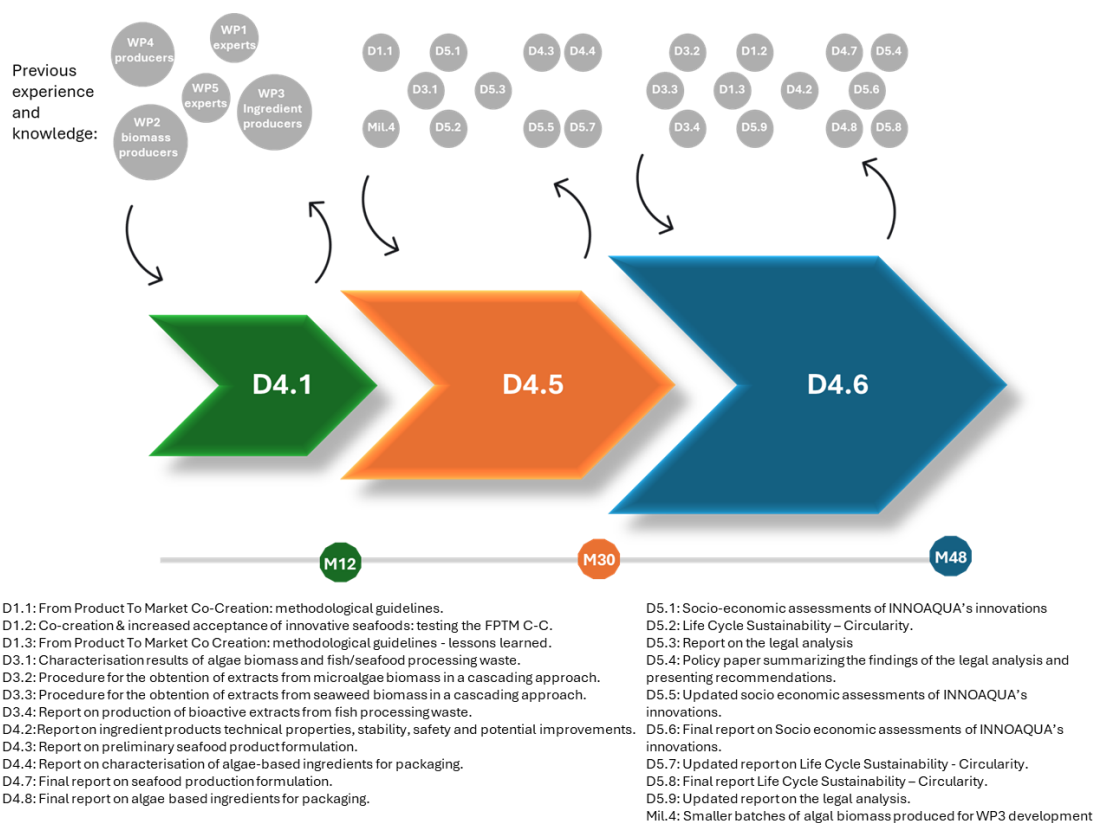


Figure 1. Relation to other INNOAQUA deliverables

2. Methodology

Task 4.1 is an overarching task centralising inputs from across a wide range of project activities and partners to ensure successful product formulations. Thus, a fluid communication among partners, some of them not taking an active role in WP4, is necessary. ALGEMY is the leader of this WP, and so, is serving as the communication link among the different parts.

Several strategies have been developed to interchange information and previous knowledge among partners, which will be shortly described in this section:

i) Initial meetings to establish the list of prototypes to produce:

The INNOAQUA Kick-off meeting served as the scenario for the first information exchange among partners. WP4 final producers (VIVA MARIS, LEITAT, ALGEMY, PESCANOVA AND ERANOVA) exposed their production capabilities and priorities regarding possible INNOAQUA's prototypes, and a first draft of a list of seafood products and packaging solutions prototypes was created. This list was modified during the following months, and a final version was approved during the 1st annual meeting in Bergen (M5). The list, which can be found below (Table 1 in Section 3), includes 7 different product categories and a total of 21 different prototypes, as well as 10 different ingredients produced from microalgae, macroalgae and fish wastes.

ii) Creation of tables to compile information:

To compile the detailed information of each product prototype and ingredient to be produced, several tables were created. These tables were shared with all the INNOAQUA partners involved in the development of the product prototypes and the extraction of the different ingredients from the biomass, as well as the different biomass producers and the regulation, sustainability and consumer acceptance experts. Each of these partners was able to include the necessary information in the tables, as well as to check and review the information provided by others. For each of the product prototypes the following information was compiled:

- i) Description of the product and attributes to assess its performance: including the serving size, quality attributes, nutritional attributes (if applicable), manufacturing requirements, expected shelf life, expected production cost, and consumer acceptance attributes;
- ii) Regulations to be considered;
- iii) Sustainability information to be considered during production.
- iv) Quantities to be produced in each of the stages (kitchen-scale, for the performance assessment test, industrial-scale...);
- v) Approximate formulation, indicating which INNOAQUA's ingredients to be used and in which quantities;
- vi) Characterization and test to be performed to the product to assess its quality and performance (sensory test, product acceptance panel, shelf life test, content of hazardous substances in packaging...).

For the ingredients and biomass, information was also compiled, including, additionally, information regarding the biomass composition and production cost and the expected extraction yield for each ingredient.

The different tables were filled in following a progressive pathway, starting always from the producer of the prototype/ingredient/biomass, and including later the feedback of the regulation, sustainability and consumer acceptance experts, as well as from other WP3 and WP4 partners. Lately, the compiled information was finally reviewed and approved by all the involved partners (M8).

- iii) **Meetings with regulatory, sustainability and consumer acceptance experts:**
Starting with the information provided in the above-mentioned tables, several meetings were scheduled between the WP4 product and ingredient producers and the regulatory, sustainability and consumer acceptance experts to discuss the requirements for each product/ingredient. Some meetings involved the whole group of WP4 partners (WP4 monthly meetings), and others were smaller, involving only a few participants. These meetings provided expertise and feedback regarding several aspects to be considered for the prototype producers, as well as input on which aspects must be developed and emphasized during the execution



of WP1 and WP5 tasks. Later on, when WP1 and WP5 will be extensively executed, the information and conclusions obtained in their tasks will serve as feedback for the re-definition of T4.1 prototype characteristics. This information will be updated in the subsequent deliverables (D4.5 and D4.6)

iv) Calculation of the ingredients and required amounts of biomass:

Once the information regarding the intended amounts to produce and the product formulation for each prototype was provided, it was possible to calculate the necessary amount of each ingredient. Considering the expected composition of each biomass and the expected extraction yield for each ingredient indicated by the biomass and ingredient producers, it was also possible to calculate the amount of each type of biomass that will be necessary for the development of the INNOAQUA project. All this information was collated with the initial estimations and planification, and the necessary readjustments in terms of biomass production will be considered.

v) Creation of a written report with all the compiled information:

Once all the tables were filled in by the different WP4 partners and WP1 and WP5 experts and the calculations performed, all the information was transferred to this written report, that describes all the requirements that each of the ingredients and prototypes of WP4 must fulfil.

vi) Review of the information by all the partners:

The written report was shared with all WP4 partners, as well as WP1, WP2, WP3 and WP5 members, both for their review and approval, but also to serve as starting point for the development of their respective tasks. The document provides information of the necessary amount of each biomass to be produce and its timing, the expectation of extraction yields and timings for the ingredient producers and timing and expected production amounts for the final product manufacturers. In addition, information regarding the quality attributes to fulfil, as well as regulatory, sustainability and consumer acceptance attributes to be considered is also



provided. Thus, it will serve as a guide for the development of a considerable number of tasks within the INNOAQUA project.

The tables that were created to compile information will be updated along the whole project duration with the results obtained, namely including information regarding biomass characterization, extraction yields once the cascade extraction processes are optimized, products nutrition values, shelf life, production costs, consumer acceptance, sustainability analysis, etc.

3. General list of the different ingredients, products and product categories

One of the goals of the INNOAQUA project is to demonstrate processing methods to obtain new innovative (algae-based) seafood products. Algae (both microalgae and seaweed) are sustainable sources of natural high-value proteins, polyunsaturated fatty acids (PUFAs), carotenoids, bioactive and functional carbohydrates, and vitamins. These components possess numerous benefits for high added value food applications widely sought after in the current market, especially as low carbon protein-rich alternative source. Thus, to achieve this goal, INNOAQUA partners expect to extract high-added value ingredients from algae biomass, as well as from fish by-products, to later use them in the formulation of different innovative seafood products and packaging solutions.

As described in the methodology section, the first activity done as part as T4.1 was to discuss and approve the general list of all the final seafood products and packaging solutions that each WP4 partner expects to produce as part of the INNOAQUA project. These products were clustered in different product categories: vegan meat analogues, vegan fish analogues, vegan powdered meals, vegan snacks, other vegan products, hybrid products and packaging solutions. A total of 21 prototypes were listed and approved, and for each of them, a partner was assigned as responsible for its development. The approved list of product prototypes can be found below, in Table 1. It is important to note that, despite choosing 21 different products, only 14 of them (12 seafood products and 2 packaging solutions) will be finally up scaled to an industrial stage. However, all WP4 partners have agreed to start the process with a larger number of products and develop all of them until the kitchen-scale stage. In future enough information regarding the product formulation, production process, product attributes and also consumer acceptance (WP1) and sustainability footprint (WP5) will be collected, making it possible to select and discard the least viable prototypes for industrialization, thus, ending the whole process with only 14 of them.

Table 1 Approved list of the different seafood and packaging prototypes to be produced as part of WP4.

Products		
Product category	Product	Producer
Vegan meat analogues	Vegan Sausages	VIVA MARIS
	Vegan Nuggets	LEITAT
Vegan fish analogues	Vegan Surimi	PESCANOVA
	Vegan fillets	PESCANOVA
	Vegan burgers	PESCANOVA
	Vegan battered/breaded fish portions	PESCANOVA
Vegan powdered meals	Shakes	ALGEMY
	Breakfast bowls	ALGEMY
	Soups	ALGEMY
Vegan snacks	Cookies	ALGEMY
	Gummy bears	ALGEMY/LEITAT
	Energy bars	ALGEMY
	Energy balls	ALGEMY
Other vegan products	Vegan Shots	VIVA MARIS
	Bread spreads	VIVA MARIS
Hybrid products	Fish&algae fillets	PESCANOVA
	Hybrid sausages	VIVA MARIS
	Hybrid nuggets	LEITAT
	Hybrid bread spreads	VIVA MARIS
Biodegradable packaging	Lidding films	ERANOVA
	Trays	ERANOVA

Table 2 Approved list of the different ingredients to be produced, indicating the biomass from which they will be obtained, as well as the consortium partner in charge.

Ingredients		
Biomass of origin	Ingredient	Partner in charge
Microalgae (NORCE): <ul style="list-style-type: none"> - <i>Chlorella sorokinina</i> - <i>Phaeodactylum trycornutum</i> - <i>Nannochloropsis oculata</i> 	Microalgae protein	ALGEMY
	Microalgae PUFA's and carotenoids rich oil	ALGEMY
	Microalgae hydrophilic vitamins	ALGEMY
	Microalgae peptides and AA's	ALGEMY/LEITAT
	Microalgae residual fraction	ALGEMY/LEITAT
Macroalgae (A4F): <ul style="list-style-type: none"> - <i>Ulva</i> - <i>Porphyra</i> - <i>Gracilaria</i> 	Macroalgae Proteins	A4F
	Macroalgae Agar	A4F
	Macroalgae Residual fraction	A4F
Fish waste: <ul style="list-style-type: none"> - Fish skins and heads from salmon (VIKINGAQUA) - Non-compliant surimi sticks (PESCANOVA) 	Fish functional FPHs	LEITAT
Microalgae and macroalgae residual fraction	Plastic resin	ERANOVA

For the formulation of each of the seafood prototypes (T4.3) and packaging solutions (T4.4), one or more than one ingredient obtained from algae (microalgae or macroalgae) or from fish

by-products will be used. These ingredients will be obtained and characterized in T4.2, from the algae and fish by-products extracts produced as part of WP3. In turn, the extracts will be obtained from different types of biomasses produced as part of WP2, and after being subjected to different cascade extraction protocols. A total of 5 different ingredients obtained from 3 different species of microalgae will be produced, as well as 3 different ingredients obtained from 3 different species of seaweed, and 1 last ingredient obtained from a fish by-product (either non-compliant surimi sticks or fish skins and heads from salmon) (the list can be found in Table 2). Thus, the whole process will be a multi-stage chain, starting from the algae and fish by-products biomass, and ending with the different seafood and packaging products (Figure 2). The whole chain will also be subsequently up scaled, starting with the first laboratory/kitchen tests and ending with the industrial production of the final products, which will be packed inside our own trays.

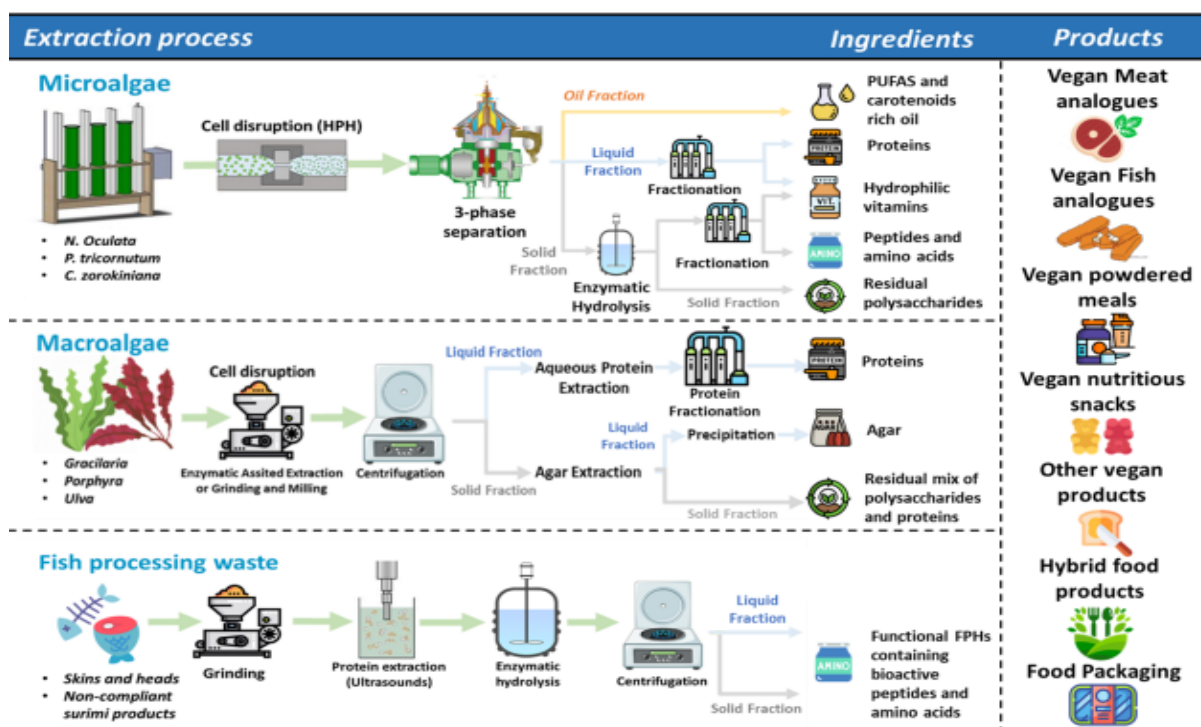


Figure 2. Summary of the multi-stage process that will end up with the obtention of the different seafood and packaging products as described in the proposal.

4. Description of the products

4.1. General description

In this section, a detailed description for each of the novel algae- or fish-based food and packaging products is provided, including the KPIs it must fulfil. For each product, the description is divided in the following sections:

- General description: a definition of the product is provided, including aspects such as the composition of the product, in terms of the different ingredients in the formulation, its nutritional information, morphological aspects of the product, etc. This section also describes the main attributes of the product and the intended consumer profile or market sector, if already established.
- Regulatory aspects and sustainability: this subsection describes the European and different local regulations that apply to the product, including details about possible necessary consultations or approval processes. Regarding sustainability, the different aspects that need to be considered for the product design and development are listed, in accordance with the final conclusions of T5.1, led by SUSTAINN. For each different product, a carbon footprint reference has been included, and later, a review of LCA, LCC and s-LCA standards of similar products, if existing, will need to be performed and will serve to establish a range of goal values in which our product must enter. This last information will be included in the next document update D4.5.

Some of this information is common for all the products. To avoid reiteration, the general description that applies to all products is included in section 4.1.1, while the specific information for each product is included in the subsequent sections.

- Essential attributes for manufacturing: in this section the product serving size and format is described. It also includes information regarding the manufacturing process that needs to be considered during the design and development of the products which may affect the format of the ingredients. A first expected production cost is estimated, even though this value will be further updated in the forthcoming deliverable updates, as this value will strongly depend on the final product formulation and production process.

- Quantities to be produced and specific amounts of required ingredients: in this section, the amount of the product that is expected to be produced in each stage of the development process (kitchen product development and industrial production) is stated. This allows to calculate which and how much of the INNOAQUA's ingredients will be necessary for this specific product, and thus, to calculate the total amount of each ingredient that must be produced.
- Methods and criteria for assessing performance describes all the tests that will be necessary to perform to the product to make sure that it fulfils the KPIs and quality criteria. It may include the test to determine the shelf-life, biological test to assess safety measurements, sensory tests, consumer acceptance studies, etc. Regarding the consumer acceptance aspects, a general introduction has been included in subsection 4.1.2 to avoid reiteration, and more specific details are provided for each of the products.

4.1.1. Regulatory and sustainability aspects

4.1.1.1 Sustainability

One of the main goals of the INNOAQUA project is to take sustainability and circular economy to the next step. From the very beginning of the seafood and packaging product development sustainability and circularity have been considered, looking in every single step to choose the most sustainable options. Thus, the sustainability aspects of the product development will not only be considered in the industrial development step, but have already been discussed for the product conceptualization, and will also take a crucial role during the kitchen-scale prototypes development. In doing so, we expect to be able to get ahead of the challenges that will arise, and to be able to plan and choose the most appropriate options in order to produce the most sustainability aligned products.

To achieve our goals in terms of sustainability, some requirements have been established. Some of them apply to all the INNOAQUA products, and so, they will be described in this subsection. Later, for each individual product, we look for specific benchmark references. The references are the existing commercial non-vegan/hybrid products that we aim to substitute,

and we look for certified environmental impacts and carbon footprints for this reference products. In doing so, we determine which would be the minimum sustainability KPI's we aim to achieve.

The main strategies and public policies in the field of sustainability and circular economy at a European level related to INNOAQUA are:

- European Green Deal (2).
 - Green Deal Industrial Plan (3).
 - Circular Economy Action Plan (4).
 - Green Deal and Packaging (5).
 - Ecodesign for Sustainable Products Regulation (6).
 - Farm to Fork Strategy (7).
 - Green Claims Directive Proposal (8).
- EU Taxonomy for Sustainable Activities (9).
 - EU Taxonomy Navigator (10).

Regarding the packaging solutions, some guidelines may be useful:

- Circular Pack Guidelines
 - Guide to promote circularity in food packaging (11).
- Criteria for sustainable packaging:
 - Packaging checklist (12).
 - Product Labels (13).
- Sustainable Packaging Coalition
 - Design for Recycled Content Guide (14)
 - CEFLEX Circular Economy for Flexible Packaging (15)

For the products sustainability requirements and actions to be performed, two main groups have been defined: the first applies to the kitchen development stage, while the second applies to the industrialization stage.

For the kitchen development stage, the general sustainability and circular economy steps to be taken into account for measuring sustainability and circularity are:

- Definition of sustainability and circular economy KPI's to be measured and to be defined as requirements for industrialization process, such as:
 - Raw materials and auxiliary materials,
 - Energy consumption and energy source,
 - Water consumption and water recirculation,
 - Waste, including:
 - Food waste,
 - Wastewater,
 - Other kind of waste;
- Measurement of the KPIs previously defined;
- Estimation of the environmental impacts (LCA estimation), including estimation of the carbon footprint. This estimation will be done for the kitchen development stage, and the data obtained will be also used for an extrapolation to the industrialization phase.
- Identification of critical parameters related to circularity-sustainability (social impact, economic impact, environmental impacts);
- Risk assessment related to environmental impacts and carbon footprint, identifying critical parameters, such as:
 - suppliers, raw materials origin, transportations, distributions approaches, end-of-life management of packaging waste, etc
- Circularity assessment estimation.

In addition, all the information compiled during the kitchen development stage will serve to estimate and define the sustainability and circularity requirements for the industrialization stage, such as:

- Energy consumption requirements,
- Water consumption requirements,
- Material requirements,
 - Recycled content of materials (for the packaging),
 - Biodegradable content of materials,
- Carbon footprint,
- Environmental impacts requirements.

Later, during the industrialization stage, the following requirements will be considered:

- Related to Sustainability-Circularity KPIs definition and measurement identified within the kitchen development stage;
- Related to parameters to measure in manufacturing digitalization processes, such as:
 - water consumption,
 - energy consumption,
 - waste generation of any kind, including but not limited to:
 - Food waste,
 - Waste water,
 - carbon footprint;
- Related to activities to carry out
 - LCA,
 - Carbon footprint calculation;
- Related to potential certifications to obtain (EPD, Carbon footprint, etc);
- Related to life cycle costs:
 - Environmental impacts related costs: carbon footprint calculation and certification costs, EPD development costs, compensation costs, etc;
- Related to Developers Sustainability / Decarbonization Strategies:
 - Example: Carbon Neutral 2040 --> consider how new products have to contribute to company's strategies (carbon footprint reduction and compensation),
 - Social Impacts on the different stakeholders along the products and ingredients development;
- Social sustainability impact estimation;
- Life cycle sustainability assessment for every product considering the concrete formulation and its whole life cycle., including:
 - Life Cycle Assessment (LCA),
 - Life Cycle Cost Assessment (LCC),
 - Social Life Cycle Assessment (SLCA).



For each specific product, sustainability performance reference to consider for any kind of products should be identified. Main references measuring sustainability performance of existing products within agrifood sector are Environmental Product Declarations.

These platforms provide information about certified environmental impacts and carbon footprint of different products that could be used as a reference. Some of the platforms have specific categories for food & beverages products, packaging, etc.

- The International EPD System (16), includes EPDs, among others, for the following category products potentially applicable to INNOAQUA scope:
 - Chemical products,
 - Food & Beverages,
 - Paper and plastic products;
- PD Norge (Norway) (17), includes EPDs, among others, for the following category products potentially applicable to INNOAQUA scope:
 - Packaging and accessories,
 - Chemicals,
 - Sea-based aquaculture infrastructure and components.

For each of the INNOAQUA products, the databases have been searched for the most adequate products to serve as comparison. The selected references will be indicated in each of the product's subsections below.

4.1.1.2 Regulation

It is also very important to have in mind, from the very beginning of the product development process, which regulations will or may apply to the said product. Next, we list the most relevant regulations that will apply to all the seafood products. Later, in each product section, we indicate if there is, additionally, any relevant regulation that applies specifically to that product. For the packaging solutions, the specific regulation that applies to them is described in the respective section.

- Novel Food Regulation. EU 2015/2283: this regulation concerns the food not consumed by humans in the EU before 15 May 1997, and thus, not automatically

approved for consumption. When it cannot be proved that a certain food was consumed in the EU before this date, it is necessary to apply for a novel food authorization before its commercialization. After an extensive study the EC decides about the authorization or refusal of the product. The novel food status of food products can be checked in the EU Novel Food Status Catalogue (18).

Some species of microalgae and seaweed are not considered novel food, as it could be proved that they have been consumed for decades in the EU, while other products such as certain extracts and oils have been approved as novel food. Regarding the algae incorporated in the INNOAQUA project, *Chlorella Sorokiniana*, *Gracilaria Gracilis*, *Ulva Intestinalis* and several species of *Porphyra* have already been listed as non-novel food, while oil from *Phaeodactylum tricornutum* is awaiting to be approved as novel food (19) and extracts from both *Phaeodactylum tricornutum* and *Nannochloropsis oculata* have received a New Dietary Ingredient Notification. In addition, it is unclear if processing an approved algae up to a certain level, makes it become novel food, or it keeps being an approved food. For these doubtful cases, it is possible to place a consultation to the competent authorities of the EU country where it may be commercialized. In the case of some of the INNOAQUA ingredients, it may become necessary.

- General food law. EC 178/2002. This regulation establishes the basic requirements for food safety in the EU. All the INNOAQUA seafood products will need to fulfil this law.
- Food Hygiene Regulation. EC 852/2004. This Regulation lays down general rules for food business operators on the hygiene of food. All the INNOAQUA products will need to fulfil this regulation.
- Microbiological criteria Commission Regulation. EC 2073/2005. This regulation sets legal microbiological criteria for a range of foods to assure food safety, and that all the INNOAQUA seafood products will need to fulfil.

- Contaminants Commission Regulation. EC 2023/915. This regulation sets maximum levels for certain contaminants in food that the INNOAQUA seafood products will need to fulfil.
- Monitoring of metals and iodine in seaweed, halophytes and products based on seaweed. Commission Recommendation 2018/464. A call for the monitoring of arsenic, cadmium, iodine, lead and mercury levels was placed. Thus, as part of the INNOAQUA project, it will be good to check the levels of these elements in the final seafood products.
- Addition of vitamins and minerals and of certain other substances to foods Regulation. EC 1925/2006. This regulation lays down general rules for the fortification of foods with vitamins, minerals and other substances. It contains a list of approved minerals and vitamins for fortification. The maximum levels of vitamins and minerals that a food can contain is not yet regulated, but it is under discussion.

In addition to the regulations listed, it will be necessary to keep updated for any possible regulation modification.

4.1.2. Consumer acceptance aspects

The evaluation of different aspects regarding the consumer acceptance will be performed along the INNOAQUA project with different tools and methods. WP1 focuses on understanding how the consumption of innovative seafoods is affected by social norms and the consumer perceptions and on the identification of market penetration strategies. As part of this WP, a co-creation process for the seafood products concept and design will be performed together with the final consumers. All this information will be obtained, among other methods, by means of interviews with consumers, surveys, etc. and will try to answer which are the motivations for a certain consumer segment to try or not to try a new seafood product, as well as which aspects are considered or relevant to them. The detailed From Product To Market Co-Creation Methodology is described in D1.1.

In addition, at the end of the product development process, and as part of the quality assessment of WP4, some consumer acceptance tests will be performed, where the final consumers will be given end-products to try and evaluate. This test will focus on sensory aspects such as the smell, taste, colour, appearance or texture, among others, and will be performed by a panel of at least 40 final consumers.

For the development of all these tasks involving consumer acceptance, a preliminary list of concepts has been prepared, which includes all the aspects that the different food producers and sociologists involved in the INNOAQUA project have considered relevant. All of them will be shortly defined here (Table 3), and in each product section, it will be explained which of them are considered especially relevant for a given product.

Table 3 List of concepts relevant for the consumer acceptance of INNOAQUA’s products

Regarding taste		
	Definition	Keywords
Sourness	Fresh flavour from organic acids	Citrus, green apple
Sweetness	Related to sweet flavour from glucose or fruity sweetness	Sugar, prune, melon
Saltiness	Salty flavours	Sea salt
Bitterness	Bitter flavours (e.g. caffeine or quinine)	Coffee, grapefruit, tannins
Umami	Strong flavour of broth associated with meat	Meat, broth, glutamate, fish sauce, smoked meat
Grassiness	Strong flavour of green related to plants	Grass, hay, green vegetables
Liquorice flavour	Flavour of liquorice	Anise, liquorice, fennel
“Sea” association	Related to smell and flavour of the sea	Sea, seaweed, coast, boat house, pier

Fruity	Related to the smell and flavour of fruits	
Aftertaste	Presence of aftertaste	
Regarding texture		
	Definition	Keywords
Chewiness	Resistance when chewing	chewy = fibrous texture
Crispiness	Quality along a continuum from crispy to soggy	crispy = good bite
Juiciness	Quality of containing juice and being enjoyable to eat	
Creaminess	Quality of being like cream or containing cream	creamy = not lumpy
Tenderness	Quality of being easy to cut or chew	
Tactile	Quality of having a surface that is pleasant or attractive to touch	
Spreadability	Quality of being easily spread over a surface	
Regarding smell and colour		
Adequacy	For vegan analogues: The smell/colour is adequately similar to the smell/colour of the original product	
Regarding the label		
Clean	Label not listing ingredients that are perceived by consumers as undesirable (e.g. preservatives, additives, artificial colours or flavours...).	
Other		
High protein content		
High energy content		
Low salt		
Low sugar		
Vitamin profile		
No preservatives		
Fruit as sweeteners		
Superfood ingredients		
Easy solubilization		
Easy to prepare		



Vegetable content
No palm oil
Low in saturated fats
High in zinc
Healthiness
Calories
Naturalness: not being processed
Ethics
Sustainability
Sourced/produced locally

4.2. Vegan Meat analogues

A meat analogue is a vegan or vegetarian product intended to replace a meat product. It often aims to resemble the meat equivalent as much as possible, not only in appearance but also in terms of flavour, texture, mouthfeel and nutritional content. Thus, the sources of protein often need to be texturized and/or extruded to resemble the meat fibrous structure.

As part as INNOAQUA project, two different vegan meat analogues have been selected: sausages and nuggets.

4.2.1. Vegan Sausages

4.2.1.1. *General description*

The goal is to produce vegan sausages using the protein and other ingredients produced as part of the INNOAQUA project. These ingredients will need to provide the analogue meat product with a solid consistency and a good vitamin and amino acid profile. This product will be developed with the lead of the partner VIVA MARIS.

The final product is expected to have a nice texture, resembling the meat sausages, and with a protein content of at least 40% of the product weight (including protein obtained from macroalgae, microalgae or a mixture of both). The product will also be enriched with the hydrophilic B vitamins obtained from microalgae, as well as with poly-unsaturated fatty acids

obtained from microalgae (a maximum of 1% of the total product weight), which will provide the final product with an excellent nutritional profile and a high energy content.



Figure 3. Image of the expected appearance of the product. Source: VIVA MARIS.

4.2.1.2. *Regulatory aspects and sustainability*

For the sustainability requirements definition, a reference consisting in pork sausages has been found (20), with a carbon footprint of 3.7 kg CO₂ eq. per kg of final product ready for consumption (including the required packaging and cooking), as a well as a LCA document about plant-based sausages (21) from Impossible Foods Inc, with a carbon footprint of 2.09 kg CO₂ eq. (frozen and uncooked). Other more general useful references could be those of veal meat (22; 23), with carbon footprints of 29.8 (fresh) and 27 kg CO₂ eq. (including cooking), respectively.

Regarding the regulatory aspects, all those listed in section 4.1.1 apply to this product.

4.2.1.3. *Essential attributes for manufacturing*

The sausages will weigh around 60g each and will be served in packages of 4 units. Thus, 240g per serving. It is expected that the manufacturing cost of each serving will be around 1.80€.

For its manufacturing, the texture and colour will be essential. It is important for the end product to not have a strong green or brownish colour, as it would disincentivize consumer acceptance. The texture is also important, as it needs to be solid and resembling that of meat-sausages. Thus, a texturization of the microalgae and seaweed protein could be necessary.

4.2.1.4. *Quantities to be produced and specific amounts of required ingredients*

As part of the INNOAQUA project, the vegan sausage will be developed following different steps. The first formulation tests will be performed in kitchen-scale to obtain the first prototype. This prototype will be characterized and tested, and if it achieves a good performance, it will be later scaled-up to industrial level and produced at larger amounts with the aim to be introduced into the market of some EU countries.

The task T4.3 “Formulation and validation of food products” starts at M20. By November of 2025 (M30), it is expected that the kitchen prototypes will have been developed. A total of 5kg of vegan sausages are expected to be produced during this period. However, the necessary ingredients will be needed by M20 to be able to perform the necessary tests and formulate the prototypes. For the accelerated shelf-life studies, which will be performed at the end of 2025, a total of 500g of the product will be necessary. Later on (during 2027; M44-48), and after industrial scaling up (if it applies), the sensory tests and consumer acceptance tests will be performed. For the first, approximately 500g of the product will be necessary, while ca. 3 kg will be needed for the later.

4.2.1.5. *Methods and criteria for assessing performance*

To assess the performance of the product kitchen prototype, and later the industrial prototype (if scaled-up), several tests will be necessary:

- Accelerated shelf-life test: it will be necessary to establish the amount of time that the product can be stored without losing its properties and the adequacy to be consumed. To do so, accelerated shelf-life test will be performed: first in the kitchen- prototype and subsequently in the final industrial prototype. It is expected for the product to have a shelf-life of at least **six months**.
- Consumer acceptance: information regarding the consumer acceptance of the products will be provided by WP1. However, as part of the assessment of the product quality, an additional sensory (taste, colour, texture, smell...) consumer acceptance evaluation will be performed as part of task T4.3. This evaluation will be performed at the end of the industrial developing stage, with a panel of at least 40 consumers.

In addition of the general consumer acceptance parameters described in section 4.1.2, the following parameters have been identified as especially relevant for the vegan sausages: **solid structure, no aftertaste, low salt content, no green or brown colour, high protein content, good bite and good smell.**

- Sensory tests: the sensory evaluation of the prototype will be performed by an expert panel, consisting of a minimum of 8 taster experts trained for this purpose. This evaluation will be conducted at the end of the industrial stage.
- General characterization, including the assessing of iodine and heavy metals content, to make sure that they are according to the regulations, as well as biological safety tests.

4.2.2. Nuggets

4.2.2.1. *General description*

The second vegan meat analogue to be developed are the vegan nuggets. This product will consist of small pieces of vegan texturized protein that will be breaded or battered and finally deep-fried or baked. They will resemble the chicken nuggets largely available in the market.

The product will need to have a high protein content (higher than 20%), as well as a vitamin enrichment. In addition, the product is expected to have a good colour (golden brown), juiciness and a fibrous texture resembling meat. To achieve these requirements, some of the ingredients produced during the INNOAQUA project will be employed, namely microalgae and macroalgae protein (up to a 30% each), microalgae peptides and AA's up to a 5% and it may also contain microalgae PUFA's (up to 1%). Depending on the quality of the INNOAQUA protein (specially in terms of colour), protein obtained from other vegan sources (e.g. soy, pea, chickpea) may be also included in the blend (less than 10%).

The development of this prototype will be led by LEITAT.



Figure 4. Vegan chicken nuggets

4.2.2.2. Regulatory aspects and sustainability

For the sustainability requirements definition, a reference consisting in chicken breast has been found (24), with a carbon footprint of 5.96 kg CO₂ eq. (packaging included). Other more general useful references could be those of veal meat (22; 23), with carbon footprints of 29.8 (fresh) and 27 (including the cooking) kg CO₂ eq, respectively.

Regarding the regulatory aspects, all those listed in section 4.1.1 apply to this product.

4.2.2.3. Essential attributes for manufacturing

The serving size for this product is estimated to be of 100g, which will correspond to six pieces of nuggets. The expected production cost for the serving is of around 3€.

To achieve a product resembling the original meat nuggets, it is very important for the protein to be texturized, to obtain a fibrous texture. A dry extrusion process will most probably be needed. In addition, the product is expected to be juicy and with a soft and non-sticky texture.

The inside colour of the final product will also be relevant, to have high consumer acceptance. Green or brownish colours, typical from algae biomass need to be avoided.

As stated in the previous section, this product will be developed by the partner LEITAT. However, LEITAT only has the capability to produce food products up to a kitchen scale. Thus, either this product won't be able to be selected for the industrial scale production, or a third party will be necessary to scale the production up to an industrial stage.

4.2.2.4. *Quantities to be produced and specific amounts of required ingredients*

As part of the INNOAQUA project, the vegan nuggets will be developed following different steps. The first formulation tests will be performed in kitchen-scale to obtain the first prototype. This prototype will be characterized and tested, and if it achieves a good performance, its later scale-up production to industrial level may be considered, even the partner in charge of this product (LEITAT) does not have the capabilities to do so.

The task T4.3 “Formulation and validation of food products” starts at M20. By November of 2025 (M30), it is expected that the kitchen prototypes will have been developed. A total of 2kg of vegan nuggets are expected to be produced during this period. However, the necessary ingredients will be needed by M20 to be able to perform the necessary tests and formulate the prototypes. For the accelerated shelf-life studies, a total of 300g of the product will be necessary. Subsequently (during 2027; M44-48), and after industrial scaling up (if it applies), the sensory tests and consumer acceptance tests would be performed. For the first, approximately 300g of the product will be necessary, while ca. 5 kg would be needed at a later stage.

4.2.2.5. *Methods and criteria for assessing performance*

To assess the performance of the product kitchen prototype, and later the industrial prototype (if scaled-up), several tests will be necessary:

- Accelerated shelf-life test: it will be necessary to establish the amount of time that the product can be stored without losing its properties and the adequacy to be consumed. To do so, accelerated shelf-life test will be performed: first in the kitchen- prototype and later, on the final industrial prototype (if produced). It is expected for the product to have a shelf-life of at least **six months**.
- Consumer acceptance: information regarding the consumer acceptance of the products will be provided by WP1. However, as part of the assessment of the product quality, an additional sensory (taste, colour, texture, smell...) consumer acceptance evaluation will be performed as part of task T4.3, **only if this product is scaled-up to industrial level**. This evaluation would be performed at the end of the industrial developing stage, with a panel of at least 40 consumers.

In addition of the general consumer acceptance parameters described in section 4.1.2, the following parameters have been identified as especially relevant for the vegan nuggets: **Good flavour, crunchiness, juiciness, no aftertaste, high protein content, fibrous texture but soft at bite.**

- Sensory tests: the sensory evaluation of the prototype will be performed by an expert panel, consisting of a minimum of 8 taster experts trained for this purpose. This evaluation will be conducted at the end of the industrial stage, **only if the product is scaled up to industrial level.**
- General characterization, including the nutritional composition, assessing of iodine and heavy metals content, to make sure that they are according to the regulations, as well as biological safety tests.

4.3. Vegan Fish analogues

A fish analogue is a vegan or vegetarian product intended to replace a fish product. It often aims to resemble the fish equivalent as much as possible, not only in appearance but also in terms of flavour, texture, mouthfeel and nutritional content. Thus, the sources of protein often need to be texturized and/or extruded to resemble the fish fibrous structure.

As part as INNOAQUA project, four different vegan fish analogues have been selected: surimi, fillets, burgers and battered/breaded portions.

4.3.1. Vegan Surimi

4.3.1.1. *General description*

A vegan crab stick analogue, made with vegan surimi will be developed using the algae protein obtained during the INNOAQUA project. In this case, we will look for a high protein product, where the texture will be fundamental. It needs to be juicy, with high moisture and a soft and a surimi stick like texture, with a certain elasticity. The flavour is expected to be as “of sea”. The leader for the development of this prototype will be PESCANOVA.

The expected nutritional attributes are a 8-10% of protein, around 1% of fat, around 12% of carbohydrates and around 0.1% of omega-3 EPA+DHA. It will have a moisture content of

around 75%. The INNOAQUA ingredients employed to achieve this nutritional composition will be microalgae protein (with a maximum of a 10%), macroalgae protein (with a maximum of 10%, Microalgae PUFA's (with a maximum of 2%) and Macroalgae Agar (with a maximum of 1%).

Regarding the design of the product, it is planned to replace the red-orange coloured band characteristic of the crab sticks with an analogous green band, to highlight the vegan and natural origin of the product. The implementation of natural pigments obtained from microalgae (e.g. chlorophylls) to provide with the green colour will be considered.



Figure 5. Original surimi sticks (left) and expected appearance of the vegan product (right). Source: PESCANOVA

4.3.1.2. *Regulatory aspects and sustainability*

For the sustainability requirements definition, a surimi reference has not been found. However, other products such as Atlantic salmon (25) (carbon footprint of 4.74 kg CO₂ eq), king salmon (26) (carbon footprint of 13.3 kg CO₂ eq) or other fish products (27) (carbon footprint of 4.999 kg CO₂ eq) can be used as reference.

Regarding the regulatory aspects, all those listed in section 4.1.1 apply to this product. In addition, as this product will want to have a gluten and lactose free claim, it must have <20 ppm of gluten to fulfil the (EU) Regulation 828/2014. Regarding lactose, there is not yet a EU regulation, but the Spanish government published a guideline which indicates a recommended level of <100 ppm of lactose (28). As this product is intended to be sold mainly in Spain, we will take this value as our reference.



4.3.1.3. Essential attributes for manufacturing

The serving size will include 4 sticks of 19g each, making a total of 76g. The expected production cost per serving is between 3 and 4€.

To achieve the desired texture for the product the algae protein will need to be dried and powdered, or a wet extrusion process may be necessary. The omega-3 fatty acids may be either dried and powdered or in a liquid format.

It is also important for the dough to be white, to resemble the original surimi sticks, and the final product must be elastic, presenting at least a 1cm elasticity. The water holding capacity of the dough and final product is also important, and it should be stable after pasteurization.

4.3.1.4. Quantities to be produced and specific amounts of required ingredients

As part of the INNOAQUA project, the vegan crab sticks will be developed following different steps. The first formulation tests will be performed in kitchen-scale to obtain the first prototype. This prototype will be characterized and tested, and if it achieves a good performance, it will be later scaled-up to industrial level and produced at larger amounts with the aim to be introduced into the market of some EU countries.

Task T4.3 “Formulation and validation of food products” starts at M20. By November of 2025 (M30), it is expected that the kitchen prototypes will have been developed. A total of 5kg of vegan crab sticks are expected to be produced during this period. However, the necessary ingredients will be needed by M20 to be able to perform the necessary tests and formulate the prototypes. For the accelerated shelf-life studies, which will be performed at the end of 2025, a total of 2kg of the product will be necessary. Subsequently (during 2027; M44-48), and after industrial scaling up (if it applies), the sensory tests and consumer acceptance tests will be performed. For the first, approximately 56 servings are necessary, while for the later, the minimum amount will be 200 servings. Thus, a total of 22kg will be produced to supply for both tests, while a **total industrial production of 100-200 kg** is planned.

4.3.1.5. Methods and criteria for assessing performance

To assess the performance of the product kitchen prototype, and later the industrial prototype, several tests will be necessary:

- Accelerated shelf-life test: it will be necessary to establish the amount of time that the product can be stored without losing its properties and the adequacy to be consumed. To do so, accelerated shelf-life test will be performed: first in the kitchen- prototype and later the final industrial prototype. It is expected for the product to have a shelf-life of at least **45 days chilled**, which will be the preferred format. If the serving format needs to be change to frozen, the shelf-life should be of at least 18 months.
- Consumer acceptance: information regarding the consumer acceptance of the products will be provided by WP1. However, as part of the assessment of the product quality, an additional sensory (taste, colour, texture, smell...) consumer acceptance evaluation will be performed as part of task T4.3. This evaluation will be performed at the end of the industrial developing stage, with a panel of at least 40 consumers. In addition of the general consumer acceptance parameters described in section 4.1.2, the following parameters have been identified as especially relevant for the vegan crab sticks: **texture like that of crab stick, juiciness, sea flavour, white colour.**
- Sensory tests: the sensory evaluation of the prototype will be performed by an expert panel, consisting of a minimum of 8 taster experts trained for this purpose. An additional product assessment panel (at least 10 members) will also validate the product. These evaluations will be conducted at the end of the industrial stage.
- General characterization, including the assessing of iodine and heavy metals content, to make sure that they are according to the regulations, as well as biological safety tests.
- Tests inside the packaging: The vegan crab sticks are one of the products that have already been selected for testing them inside the packaging developed at T4.4. Thus, part of the industrial product production will be packed and employed for the shelf-life studies inside the packaging and the migration tests. More details will be provided in the packaging section below.

4.3.2. Filets

4.3.2.1. General description

The second vegan fish analogue product that will be developed as part of the INNOAQUA project is a vegan fish filet. This will be an analogue to a fish filet, without any covering and designed to be cooked in the grill or in the oven.

In this case, we will look for a high protein product, where the texture will be fundamental. It needs to be juicy and resembling as much as possible that of natural fish, as there won't be any breaded or battered layer to mask the differences. The colour will also be fundamental and needs to resemble the original version. Thus, white colour will be the desired option, but if it turns out impossible to produce a pure-white protein, other options would be to take as a reference salmon or trout fillets. The flavour is also important and is expected to be as “of sea”. The leader for the development of this prototype will be PESCANOVA.

The expected nutritional attributes are around 15% of protein, around 2-4% of fat and around 0.4% of omega-3 EPA+DHA. It will have a moisture content of around 78-82%. The INNOAQUA ingredients employed to achieve this nutritional composition will be microalgae protein (with a maximum of a 10%), macroalgae protein (with a maximum of 10%), Microalgae PUFA's (with a maximum of 2%), microalgae vitamins (around 0.1%) and Macroalgae Agar (with a maximum of 1%).



Figure 6. Image of the fish original product to be replaced. Source: PESCANOVA.

4.3.2.2. Regulatory aspects and sustainability

For the sustainability requirements definition, products such as Atlantic salmon (25) (carbon footprint of 4.74 kg CO₂ eq), king salmon (26) (carbon footprint of 13.3 kg CO₂ eq) or other fish products (27), with a carbon footprint of 4.999 kg CO₂ eq can be used as reference.

Regarding the regulatory aspects, all those listed in section 4.1.1 apply to this product. In addition, as this product will want to have a gluten and lactose free claim, it must have <20 ppm of gluten to fulfil the (EU) Regulation 828/2014. Regarding lactose, there is not yet a EU regulation, but the Spanish government published a guideline which indicates a recommended level of <100 ppm of lactose (28). As this product is intended to be sold mainly in Spain, we will take this value as our reference.

4.3.2.3. Essential attributes for manufacturing

The serving size will be a portion of 80g. The expected production cost per serving is between 3 and 4€.

In order to achieve the desired texture for the product the algae protein will need to be dried and powdered, or a wet extrusion process may be necessary. The omega-3 fatty acids may be either dried and powdered or in a liquid format.

It is also important for the dough to be white, to resemble the original fish fillets, or if it is not possible, we will look for a pink colour to resemble salmon fillets. The water holding capacity of the dough and final product is also important, and it should be stable in freeze.

4.3.2.4. Quantities to be produced and specific amounts of required ingredients

As part of the INNOAQUA project, the vegan fish analogue filets will be developed following different steps. The first formulation tests will be performed in kitchen-scale to obtain the first prototype. This prototype will be characterized and tested, and if it achieves a good performance, it will be later scaled-up to industrial level and produced at larger amounts with the aim to be introduced into the market of some EU countries.

Task T4.3 “Formulation and validation of food products” starts at M20. By November of 2025 (M30), it is expected that the kitchen prototypes will have been developed. A total of 5kg of vegan fish fillets are expected to be produced during this period. However, the necessary

ingredients will be needed by M20 to be able to perform the necessary tests and formulate the prototypes. For the accelerated shelf-life studies, which will be performed at the end of 2025, a total of 2kg of the product will be necessary. Later (during 2027; M44-48), and after industrial scaling up (if it applies), the sensory tests and consumer acceptance tests will be performed. For the first, approximately 56 servings are necessary, while for the later, the minimum amount will be 200 servings. Thus, a total of 22kg will be produced to supply for both tests, while a total industrial production of 100-200 kg is planned.

4.3.2.5. Methods and criteria for assessing performance

To assess the performance of the product kitchen prototype, and later the industrial prototype, several tests will be necessary:

- Accelerated shelf-life test: it will be necessary to establish the amount of time that the product can be stored without losing its properties and the adequacy to be consumed. To do so, accelerated shelf-life test will be performed: first in the kitchen- prototype and later on the final industrial prototype. It is expected for the product to have a shelf-life of at least **18 months frozen at -18°C**.
- Consumer acceptance: information regarding the consumer acceptance of the products will be provided by WP1. However, as part of the assessment of the product quality, an additional sensory (taste, colour, texture, smell...) consumer acceptance evaluation will be performed as part of task T4.3. This evaluation will be performed at the end of the industrial developing stage, with a panel of at least 40 consumers. In addition of the general consumer acceptance parameters described in section 4.1.2, the following parameters have been identified as especially relevant for the vegan fish fillets: **texture like fish fillets, juiciness, sea flavour, white colour (or pink if it is a salmon fillet analogue)**.
- Sensory tests: the sensory evaluation of the prototype will be performed by an expert panel, consisting of a minimum of 8 taster experts trained for this purpose. An additional product assessment panel (at least 10 members) will also validate the product. These evaluations will be conducted at the end of the industrial stage.

- General characterization, including the assessing of iodine and heavy metals content, to make sure that they are according to the regulations, as well as biological safety tests.
- Tests inside the packaging: The vegan fish fillets are one of the products that have already been selected for testing them inside the packaging developed at T4.4. Thus, part of the industrial product production will be packed and employed for the shelf-life studies inside the packaging and the migration tests. More details will be provided in the packaging section below.

4.3.3. Burgers

4.3.3.1. *General description*

The next vegan fish analogue product that will be developed as part of the INNOAQUA project is a vegan fish burger. This will be an analogue to a fish burger, without any coverage and designed to be cooked in the grill.

In this case, we will look for a high protein product, where the texture will be fundamental. It needs to be juicy and resembling as much as possible that of natural fish burgers, as there won't be any breaded or battered layer to mask the differences. The flavour is also important and is expected to be as "of sea". The leader for the development of this prototype will be PESCANOVA.

The expected nutritional attributes are around 15% of protein, around 2-4% of fat, around 3-5% of carbohydrates and around 0.3% of omega-3 EPA+DHA. It will have a moisture content of around 78-82%. The INNOAQUA ingredients employed to achieve this nutritional composition will be microalgae protein (with a maximum of a 10%), macroalgae protein (with a maximum of 10%), Microalgae PUFA's (with a maximum of 1%), microalgae vitamins (around 0.1%) and Macroalgae Agar (with a maximum of 1%). The addition of seaweed biomass with a total content of up to a 20% will also be considered.



Figure 7. Image of the original fish product to be replaced. Source: PESCANOVA.

4.3.3.2. *Regulatory aspects and sustainability*

For the sustainability requirements definition, fish burger references were not found, but a meat burger (29) reference, with a carbon footprint of 12.2 kg CO₂ eq (including cooking) has been found and can be used as reference. Other products such as Atlantic salmon (25) (with a carbon footprint of 4.74 kg CO₂ eq), king salmon (26) (with a carbon footprint of 13.3 kg CO₂ eq) or other fish products (27) (with a carbon footprint of 4.999 kg CO₂ eq) can be used as well.

Regarding the regulatory aspects, all those listed in section 4.1.1 apply to this product.

4.3.3.3. *Essential attributes for manufacturing*

The serving size will be a portion of 80g. The expected production cost per serving is between 3 and 4€.

To achieve the desired texture for the product the algae protein will need to be dried and powdered, or a wet extrusion process may be necessary. The omega-3 fatty acids may be either dried and powdered or in a liquid format.

It is also important for the dough to be white, to resemble the original fish burgers, or if it is not possible, we will look for a pink colour to resemble salmon fillets. The water holding capacity of the dough and final product is also important, and it should be stable in frozen temperatures.

4.3.3.4. *Quantities to be produced and specific amounts of required ingredients*

As part of the INNOAQUA project, the vegan fish burgers will be developed following different steps. The first formulation tests will be performed in kitchen-scale to obtain the first prototype. This prototype will be characterized and tested, and if it achieves a good performance, it will be later scaled-up to industrial level and produced at larger amounts with the aim to be introduced into the market of some EU countries.

Task T4.3 “Formulation and validation of food products” starts at M20. By November of 2025 (M30), it is expected that the kitchen prototypes will have been developed. A total of 5kg of vegan fish burgers are expected to be produced during this period. However, the necessary ingredients will be needed by M20 to be able to perform the necessary tests and formulate the prototypes. For the accelerated shelf-life studies, which will be performed at the end of 2025, a total of 2kg of the product will be necessary. Later (during 2027; M44-48), and after industrial scaling up (if it applies), the sensory tests and consumer acceptance tests will be performed. For the first, approximately 56 servings are necessary, while for the later, the minimum amount will be 200 servings. Thus, a total of 22kg will be produced to supply for both tests, while a total industrial production of 100-200 kg is planned.

4.3.3.5. *Methods and criteria for assessing performance*

To assess the performance of the product kitchen prototype, and later the industrial prototype, several tests will be necessary:

- Accelerated shelf-life test: it will be necessary to establish the amount of time that the product can be stored without losing its properties and the adequacy to be consumed. To do so, accelerated shelf-life test will be performed: first in the kitchen- prototype and later the final industrial prototype. It is expected for the product to have a shelf-life of at least **18 months frozen at -18°C**.
- Consumer acceptance: information regarding the consumer acceptance of the products will be provided by WP1. However, as part of the assessment of the product quality, an additional sensory (taste, colour, texture, smell...) consumer acceptance evaluation will be performed as part of task T4.3. This evaluation will be performed at the end of the industrial developing stage, with a panel of at least 40 consumers.

In addition of the general consumer acceptance parameters described in section 4.1.2, the following parameters have been identified as especially relevant for the vegan fish fillets: **texture like fish burgers, juiciness, sea flavour, white colour (or pink if it is a salmon burger analogue).**

- Sensory tests: the sensory evaluation of the prototype will be performed by an expert panel, consisting of a minimum of 8 taster experts trained for this purpose. An additional product assessment panel (at least 10 members) will also validate the product. These evaluations will be conducted at the end of the industrial stage.
- General characterization, including the assessing of iodine and heavy metals content, to make sure that they are according to the regulations, as well as biological safety tests.

4.3.4. Battered/ Breaded fish analogues

4.3.4.1. *General description*

The last vegan fish analogue product that will be developed as part of the INNOAQUA project is a battered or breaded fish analogue. This will be an analogue fish portion with a coverage that may be either a battering or a breading. It is expected that the coverage will contribute with 45% of the weigh while the filling will represent the 55%. The portions may be either small, to produce nuggets or larger, to produce battered/breaded filets. The leader for the development of this prototype will be PESCANOVA.

In this case, we will look for a high protein product, where the texture will be fundamental. The filling is expected to be similar to that of the vegan fish filet analogues described above. It needs to be juicy and resembling as much as possible that of natural fish. The colour will also be important and needs to resemble the original version. Thus, white colour will be the desired option for the filling, but if it turns out impossible to produce a pure-white protein, other options would be to take as a reference salmon or trout portions. The flavour is also important and is expected to be as “of sea”.

The expected nutritional attributes are around 15% of protein, around 10% of fat, 8-10% of carbohydrates and around 0.3% of omega-3 EPA+DHA. It will have a moisture content of

around 78-82%. The INNOAQUA ingredients employed to achieve this nutritional composition will be microalgae protein (with a maximum of a 5%), macroalgae protein (with a maximum of 5%), Microalgae peptides and AA's (up to 1%), Microalgae PUFA's (with a maximum of 1%) and Macroalgae Agar (with a maximum of 1%). The incorporation of some microalgae residual fraction may also be considered, in amounts lower than 5%.



Figure 8. Image of the original fish product to be replaced. Source: PESCANOVA.

4.3.4.2. *Regulatory aspects and sustainability*

For the sustainability requirements definition, products such as Atlantic salmon (25) (with a carbon footprint of 4.74 kg CO₂ eq), king salmon (26) (with a carbon footprint of 13.3 kg CO₂ eq) or other fish products (27) (with a carbon footprint of 4.999 kg CO₂ eq) can be used as references.

Regarding the regulatory aspects, all those listed in section 4.1.1 apply to this product.

4.3.4.3. *Essential attributes for manufacturing*

The serving size will be a portion of 100g (in the case of breaded/battered fillets) or 5-6 portions with a total weight of 100g. The expected production cost per serving is between 3 and 4€.

To achieve the desired texture for the product the algae protein will need to be dried and powdered, or a wet extrusion process may be necessary. The omega-3 fatty acids may be either dried and powdered or in a liquid format.

It is also important for the dough to be white, to resemble the original battered/breaded fish portions. The water holding capacity of the dough and final product is also important, and it should be stable in freeze.

4.3.4.4. Quantities to be produced and specific amounts of required ingredients

As part of the INNOAQUA project, the vegan fish burgers will be developed following different steps. The first formulation tests will be performed in kitchen-scale to obtain the first prototype. This prototype will be characterized and tested, and if it achieves a good performance, it will be later scaled-up to industrial level and produced at larger amounts with the aim to be introduced into the market of some EU countries.

Task T4.3 “Formulation and validation of food products” starts at M20. By November of 2025 (M30), it is expected that the kitchen prototypes will have been developed. A total of 5kg of vegan fish burgers are expected to be produced during this period. However, the necessary ingredients will be needed by M20 to be able to perform the necessary tests and formulate the prototypes. For the accelerated shelf-life studies, which will be performed at the end of 2025, a total of 2kg of the product will be necessary. Later (during 2027; M44-48), and after industrial scaling up (if it applies), the sensory tests and consumer acceptance tests will be performed. For the first, approximately 56 servings are necessary, while for the later, the minimum amount will be 200 servings. Thus, a total of 25kg will be produced to supply for both tests, while a total industrial production of 100-200 kg is planned.

4.3.4.5. Methods and criteria for assessing performance

To assess the performance of the product kitchen prototype, and later the industrial prototype, several tests will be necessary:

- Accelerated shelf-life test: it will be necessary to establish the amount of time that the product can be stored without losing its properties and the adequacy to be consumed. To do so, accelerated shelf-life test will be performed: first in the kitchen- prototype

and later on the final industrial prototype. It is expected for the product to have a shelf-life of at least **18 months frozen at -18°C**.

- Consumer acceptance: information regarding the consumer acceptance of the products will be provided by WP1. However, as part of the assessment of the product quality, an additional sensory (taste, colour, texture, smell...) consumer acceptance evaluation will be performed as part of task T4.3. This evaluation will be performed at the end of the industrial developing stage, with a panel of at least 40 consumers.

In addition of the general consumer acceptance parameters described in section 4.1.2, the following parameters have been identified as especially relevant for the vegan battered/breaded fish portions: **texture like fish portions, juiciness, sea flavour, white colour (or pink if it is a battered/breaded salmon portion analogue)**.

- Sensory tests: the sensory evaluation of the prototype will be performed by an expert panel, consisting of a minimum of 8 taster experts trained for this purpose. An additional product assessment panel (at least 10 members) will also validate the product. These evaluations will be conducted at the end of the industrial stage.
- General characterization, including the assessing of iodine and heavy metals content, to make sure that they are according to the regulations, as well as biological safety tests.

4.4. Vegan powdered meals

This product category contains three different vegan products that will be commercialized in powder format and will need to be mixed with water or milk for their consumption. The three selected products are: a protein shake, a breakfast bowls and a soup.

4.4.1. Shakes

4.4.1.1. *General description*

This product will consist in a dried powder mix made of one or more protein sources, that once mixed with water or (vegan) milk will become a beverage. Protein will be the main component and the powder will also include cocoa and/or lyophilized fruits and may be enriched with vitamins and minerals. ALGEMY will lead the development of this product.

The main consumers target for this product are sporty people and in general, people looking for a healthy protein supplementation, and especially those following a vegan diet. Thus, the main goal of the product will be to supply a high amount of vegan protein (around 60% in weight), but also for it to be of high nutritional value. Thus, we will look for a good amino acid profile, and the possible supplementation with vitamins, minerals and antioxidants, all of them coming from INNOAQUA's ingredients. With the supplementation of vitamins, we would look for the product to provide a high % of the recommended daily B-group vitamin intake, as they are often deficient in vegan diets.

Other important nutritional parameters will be the low sugar, and in general, carbohydrates content, a clean label and a product that can be perceived as healthy and with a good nutritional profile. The shakes will also need to be appetizing, with a good flavour and texture. For the texture, the solubility of the product in the liquid of choice (water or (vegan) milk) should be fast and excellent, resulting in a creamy product with no lumps.

The INNOAQUA ingredients formulated in this product will be microalgae protein, in an amount of around 80%, hydrophilic vitamins (less than 0.1%) and microalgae peptides and amino acids (less than 5%).



Figure 9. Image of a protein powdered shake, an example of the original product to be replaced.

4.4.1.2. Regulatory aspects and sustainability

For this product it was not possible to find a comparable reference in terms of sustainability.

Regarding the regulatory aspects, all those listed in section 4.1.1 apply to this product.

4.4.1.3. Essential attributes for manufacturing

The serving will consist of 30-40g of the powder, which will need to be mixed with 200-250 mL of water or (vegan) milk to prepare the protein shake. The production cost of one serving should be below 1.5€.

The manufacturing of the product will consist only in the blending of the different ingredients. So, all of them will need to be provided in a dried and powdered format.

4.4.1.4. Quantities to be produced and specific amounts of required ingredients

As part of the INNOAQUA project, the vegan protein shakes will be developed following different steps. The first formulation tests will be performed in kitchen-scale to obtain the first prototype. This prototype will be characterized and tested, and if it achieves a good performance, it will be later scaled-up to industrial level and produced at larger amounts with the aim to be introduced into the market of some EU countries.

Task T4.3 “Formulation and validation of food products” starts at M20. By November of 2025 (M30), it is expected that the kitchen prototypes will have been developed. A total of 1kg of vegan powdered shakes is expected to be produced during this period. However, the necessary ingredients will be needed by M20 to be able to perform the necessary tests and formulate the prototypes. For the accelerated shelf-life studies, which will be performed at the end of 2025, a total of 300g of the product will be necessary. Later (during 2027; M44-48), and after industrial scaling up (if it applies), the sensory tests and consumer acceptance tests will be performed. For the first, approximately 56 servings are necessary, while for the later, the minimum amount will be 200 servings. Thus, a total of 8 kg will be produced to supply for both tests, while a minimum industrial production of 15 kg is expected.

4.4.1.5. Methods and criteria for assessing performance

To assess the performance of the product kitchen prototype, and later the industrial prototype, several tests will be necessary:

- Accelerated shelf-life test: it will be necessary to establish the amount of time that the product can be stored without losing its properties and the adequacy to be consumed. To do so, accelerated shelf-life test will be performed: first in the kitchen- prototype and later the final industrial prototype. It is expected for the product to have a shelf-life of at least **1 year**.
- Consumer acceptance: information regarding the consumer acceptance of the products will be provided by WP1. However, as part of the assessment of the product quality, an additional sensory (taste, colour, texture, smell...) consumer acceptance evaluation will be performed as part of task T4.3. This evaluation will be performed at the end of the industrial developing stage, with a panel of at least 40 consumers. In addition of the general consumer acceptance parameters described in section 4.1.2, the following parameters have been identified as especially relevant for the vegan powdered shakes: **Good flavour, texture (creamy, not lumpy), high protein (vegan and avoiding soy), easy solubilization, low sugar, vitamin profile (B12 for vegan). No preservatives. Clean label.**
- Sensory tests: the sensory evaluation of the prototype will be performed by an expert panel, consisting of a minimum of 8 taster experts trained for this purpose. These evaluations will be conducted at the end of the industrial stage.
- General characterization, including the assessing of iodine and heavy metals content, to make sure that they are according to the regulations, as well as biological safety tests.

4.4.2. Breakfast bowls

4.4.2.1. *General description*

The second vegan powdered meal that will be developed is a breakfast bowl. It will consist of a dried powder mix made of oatmeal, fruits (and seeds) and rich in protein that once mixed with water, (vegan) milk or (vegan) yogurt will become a tasty cream perfect for breakfast. ALGEMY will lead the development of this product.

This product is designed to target the general public, but especially those following a vegan diet and/or looking for a healthy product. Thus, the main goal of the product will be to supply a high amount of vegan protein (around 20-30% in weight) as well as being highly energetic (>350kcal/100g). The protein will need to be of high quality, providing a good amino acid profile. The nutritional profile of the product will be complemented with the presence of at least a 10% of fibre, and the supplementation with vitamins and minerals obtained from the INNOAQUA ingredients. With the supplementation of vitamins, we would look for the product to provide a high % of the recommended daily B-group vitamin intake, as they are often deficient in vegan diets.

Other important nutritional parameters will be the minimization of added sugars, a clean label and a product that can be perceived as healthy and with a good nutritional profile. The breakfast bowls will also need to be appetizing, with a good fruity flavour and texture. For the texture, the solubility of the product in the liquid of choice (water, (vegan) milk or (vegan) yogurt) should be fast and excellent, resulting in a creamy product with no lumps.

The INNOAQUA ingredients formulated in this product will be microalgae protein, with a maximum amount of 30%, hydrophilic vitamins (less than 0.1%) and microalgae peptides and amino acids (less than 5%), which will be complemented with fruit, oatmeal and/or seeds.



Figure 10. Example of a breakfast bowl product. Source: foodspring

4.4.2.2. Regulatory aspects and sustainability

For the sustainability requirements definition, a breakfast bowl reference was not found. The closest references found were a granola with nuts and chocolate (carbon footprint of 1.96 kg CO₂ eq including packaging) (30) and an apple pure (0.593 kg CO₂ eq in a steel drum packaging format, 0.363 kg CO₂ eq in bulk packaging format)(31).

Regarding the regulatory aspects, all those listed in section 4.1.1 apply to this product.

4.4.2.3. Essential attributes for manufacturing

The serving will consist of 50-100 g of the powder, which will need to be mixed with 100-150 mL of water or (vegan) milk or yogurt to prepare the breakfast bowl. The production cost of one serving should be below 1.25 €.

The manufacturing of the product will consist only in the blending of the different ingredients. So, all of them will need to be provided in a dried and powdered format.

4.4.2.4. Quantities to be produced and specific amounts of required ingredients

As part of the INNOAQUA project, the vegan breakfast bowls will be developed following different steps. The first formulation tests will be performed in kitchen-scale to obtain the first prototype. This prototype will be characterized and tested, and if it achieves a good performance, it will be later scaled-up to industrial level and produced at larger amounts with the aim to be introduced into the market of some EU countries.

The task T4.3 “Formulation and validation of food products” starts at M20. By November of 2025 (M30), it is expected that the kitchen prototypes will have been developed. A total of 1kg of vegan breakfast bowls is expected to be produced during this period. However, the necessary ingredients will be needed by M20 to be able to perform the necessary tests and formulate the prototypes. For the accelerated shelf-life studies, which will be performed at the end of 2025, a total of 500g of the product will be necessary. Later (during 2027; M44-48), and after industrial scaling up (if it applies), the sensory tests and consumer acceptance tests will be performed. For the first, approximately 56 servings are necessary, while for the later, the minimum amount will be 200 servings. Thus, a total of 25kg will be produced to supply for both tests, while a minimum industrial production of 50 kg is expected.

4.4.2.5. *Methods and criteria for assessing performance*

To assess the performance of the product kitchen prototype, and later the industrial prototype, several tests will be necessary:

- Accelerated shelf-life test: it will be necessary to establish the amount of time that the product can be stored without losing its properties and the adequacy to be consumed. To do so, accelerated shelf-life test will be performed: first in the kitchen- prototype and later the final industrial prototype. It is expected for the product to have a shelf-life of at least **1 year**.
- Consumer acceptance: information regarding the consumer acceptance of the products will be provided by WP1. However, as part of the assessment of the product quality, an additional sensory (taste, colour, texture, smell...) consumer acceptance evaluation will be performed as part of task T4.3. This evaluation will be performed at the end of the industrial developing stage, with a panel of at least 40 consumers. In addition of the general consumer acceptance parameters described in section 4.1.2, the following parameters have been identified as especially relevant for the vegan breakfast bowls: **Good flavour, high energy and high protein, no sugar (fruits as sweeteners), presence of superfood ingredients (microalgae, açai, turmeric...).** **Easy solubilization. Clean label.**
- Sensory tests: the sensory evaluation of the prototype will be performed by an expert panel, consisting of a minimum of 8 taster experts trained for this purpose. These evaluations will be conducted at the end of the industrial stage.
- General characterization, including the assessing of iodine and heavy metals content, to make sure that they are according to the regulations, as well as biological safety tests.

4.4.3. Soups

4.4.3.1. *General description*

The last of the vegan powdered products that we will develop is a soup. In this case it will consist of a dried powder rich in protein and vegetables (powdered on in small pieces) that

once mixed in water will become a vegan soup. ALGEMY will lead the development of this product.

This product is designed to target the general public, but especially for those people following a vegan diet. The main goal of the product will be to supply a high amount of vegan protein (more than 40% in weight), but also for it to be of high nutritional value. Thus, we will look for a good amino acid profile, and the possible supplementation with vitamins, minerals and antioxidants, coming from INNOAQUA's ingredients as well of the different vegetables included in the soup. With the supplementation of vitamins, we would look for the product to provide a high % of the recommended daily B-group vitamin intake, as they are often deficient in vegan diets.

Other important nutritional parameters will be the low salt content, a clean label and a product that can be perceived as healthy and with a good nutritional profile. The soups will also need to be appetizing, with a good flavour and texture. For the texture, the solubility of the product in water should be fast and excellent, resulting in a product with no lumps.

The INNOAQUA ingredients formulated in this product will be microalgae protein, in an amount of a maximum of 60%, hydrophilic vitamins (less than 0.1%) and microalgae peptides and amino acids (less than 5%).



Figure 11. Image of a protein powdered soup, an example of the original product to be replaced.

4.4.3.2. Regulatory aspects and sustainability

Sustainability references for powdered soups could not be found.

Regarding the regulatory aspects, all those listed in section 4.1.1 apply to this product.

4.4.3.3. Essential attributes for manufacturing

The serving will consist of 20-30 g of the powder, which will need to be mixed with 200-250 mL of water to prepare the soup. The production cost of one serving should be below 1€.

The manufacturing of the product will consist only in the blending of the different ingredients. So, all of them will need to be provided in a dried format, either powdered or in small pieces (for the vegetables).

4.4.3.4. Quantities to be produced and specific amounts of required ingredients

As part of the INNOAQUA project, the vegan powdered soups will be developed following different steps. The first formulation tests will be performed in kitchen-scale to obtain the first prototype. This prototype will be characterized and tested, and if it achieves a good performance, it will be later scaled-up to industrial level and produced at larger amounts with the aim to be introduced into the market of some EU countries.

The task T4.3 “Formulation and validation of food products” starts at M20. By November of 2025 (M30), it is expected that the kitchen prototypes will have been developed. A total of 1kg of vegan powdered soup is expected to be produced during this period. However, the necessary ingredients will be needed by M20 to be able to perform the necessary tests and formulate the prototypes. For the accelerated shelf-life studies, which will be performed at the end of 2025, a total of 300g of the product will be necessary. Later (during 2027; M44-48), and after industrial scaling up (if it applies), the sensory tests and consumer acceptance tests will be performed. For the first, approximately 56 servings are necessary, while for the later, the minimum amount will be 200 servings. Thus, a total of 8kg will be produced to supply for both tests, while a minimum industrial production of 15 kg is expected.

4.4.3.5. Methods and criteria for assessing performance.

To assess the performance of the product kitchen prototype, and later the industrial prototype, several tests will be necessary:

- Accelerated shelf-life test: it will be necessary to establish the amount of time that the product can be stored without losing its properties and the adequacy to be consumed. To do so, accelerated shelf-life test will be performed: first in the kitchen- prototype and later the final industrial prototype. It is expected for the product to have a shelf-life of at least **1 year**.
- Consumer acceptance: information regarding the consumer acceptance of the products will be provided by WP1. However, as part of the assessment of the product quality, an additional sensory (taste, colour, texture, smell...) consumer acceptance evaluation will be performed as part of task T4.3. This evaluation will be performed at the end of the industrial developing stage, with a panel of at least 40 consumers. In addition of the general consumer acceptance parameters described in section 4.1.2, the following parameters have been identified as especially relevant for the vegan breakfast bowls: **Good flavour, good solubility (easy to prepare), low in salt, high in protein and vitamins, high vegetables content. Clean label.**
- Sensory tests: the sensory evaluation of the prototype will be performed by an expert panel, consisting of a minimum of 8 taster experts trained for this purpose. These evaluations will be conducted at the end of the industrial stage.
- General characterization, including the assessing of iodine and heavy metals content, to make sure that they are according to the regulations, as well as biological safety tests.

4.5. Vegan snacks

This category comprises a group of vegan products of small size conceived to be eaten between meals, and which do not require any cooking or preparation by the consumer. Four different vegan snacks will be developed: cookies, gummy bears, energy bars and energy balls.

4.5.1. Cookies

4.5.1.1. *General description*

The first product is a cookie. It will be a sweet vegan snack with solid consistency and enriched with vitamins and proteins. It may also contain nuts, fruit, different flours, chocolate, etc. The product development will be led by ALGEMY.

It is intended to provide a high energy peak, and so, the consumer target for this product are sporty people looking for some energy before or after high activity. To achieve so, the product will contain a high amount of protein (25-35%), which will come from the INNOAQUA microalgae. In addition, and to provide a more interesting nutritional profile, the cookies will be also enriched with microalgae hydrophilic vitamins (less than 1%) and microalgae PUFA's and carotenoid rich oils (less than 0.1%).

The texture may be soft or crunchy and the sweetener will be fruits, sugar and/or artificial sweeteners.



Figure 12. Image of a protein cookie, an example of the original product to be replaced. Source: Body&Fit.

4.5.1.2. *Regulatory aspects and sustainability*

For the sustainability requirements definition, the vegan cookies would be compared with the following references: different chocolate cookies (32; 33), with carbon footprints of 2.90 and

2.96 kg CO₂ eq, respectively, and salty biscuits (34; 35), with carbon footprints of 2.38 and 2.04 kg CO₂ eq, respectively.

Regarding the regulatory aspects, all those listed in section 4.1.1 apply to this product.

4.5.1.3. Essential attributes for manufacturing

The serving will consist of big cookies of 50 g each. The production cost of one cookie should be below 1.25€.

For the manufacturing the protein will need to be in dried and powdered form.

4.5.1.4. Quantities to be produced and specific amounts of required ingredients

As part of the INNOAQUA project, the vegan cookies will be developed following different steps. The first formulation tests will be performed in kitchen-scale to obtain the first prototype. This prototype will be characterized and tested, and if it achieves a good performance, it will be later scaled-up to industrial level and produced at larger amounts with the aim to be introduced into the market of some EU countries.

The task T4.3 “Formulation and validation of food products” starts at M20. By November of 2025 (M30), it is expected that the kitchen prototypes will have been developed. A total of 1kg of cookies is expected to be produced during this period. However, the necessary ingredients will be needed by M20 to be able to perform the necessary tests and formulate the prototypes. For the accelerated shelf-life studies, which will be performed at the end of 2025, a total of 500g of the product will be necessary. Later (during 2027; M44-48), and after industrial scaling up (if it applies), the sensory tests and consumer acceptance tests will be performed. For the first, approximately 56 servings are necessary, while for the later, the minimum amount will be 200 servings. Thus, a total of 15kg will be produced to supply for both tests, while a minimum industrial production of 30 kg is expected.

4.5.1.5. Methods and criteria for assessing performance

To assess the performance of the product kitchen prototype, and later the industrial prototype, several tests will be necessary:

- Accelerated shelf-life test: it will be necessary to establish the amount of time that the product can be stored without losing its properties and the adequacy to be consumed.

To do so, accelerated shelf-life test will be performed: first in the kitchen- prototype and later the final industrial prototype. It is expected for the product to have a shelf-life of at least **6 months**.

- Consumer acceptance: information regarding the consumer acceptance of the products will be provided by WP1. However, as part of the assessment of the product quality, an additional sensory (taste, colour, texture, smell...) consumer acceptance evaluation will be performed as part of task T4.3. This evaluation will be performed at the end of the industrial developing stage, with a panel of at least 40 consumers.

In addition of the general consumer acceptance parameters described in section 4.1.2, the following parameters have been identified as especially relevant for the protein rich cookies: **Sweet, no palm oil, low in saturated fats. Clean label**

- Sensory tests: the sensory evaluation of the prototype will be performed by an expert panel, consisting of a minimum of 8 taster experts trained for this purpose. These evaluations will be conducted at the end of the industrial stage.
- General characterization, including the assessing of iodine and heavy metals content, to make sure that they are according to the regulations, as well as biological safety tests.

4.5.2. Gummy bears

4.5.2.1. *General description*

This product is a sweet vegan snack consisting in small jelly candy made with agar and fruit and enriched with vitamins and omega-3. The product development will be led by ALGEMY and LEITAT.

It is intended to have a very good flavour (sweet and fruity) and to be appetizing, while serving as vitamin and omega-3 supplement. Thus, it is expected to have a good vitamin profile and that a single serving could provide a high percentage of the recommended daily intake of most B group vitamins. The vitamins will be obtained from the INNOAQUA microalgae hydrophilic vitamins ingredient (around 5% of the product weight). As this ingredient is also expected to be rich in minerals, the vegan gummy bears may also have a very good mineral profile. In a

similar way, it would be desirable to incorporate some of the INNOAQUA microalgae PUFA's and carotenoids rich oil (around 0.5%), as it would provide a natural and vegan source of omega-3. However, it will be necessary to test if the oil will be easily mixed with the jelly structure of the product or not.

The texture will be tender and chewy and will be provided by the agar obtained from the INNOAQUA macroalgae (up to 13% of the product weight). So, the product will be vegan. The colour and flavour will be provided by natural fruit juice.



Figure 13. Image of omega-3 rich gummy bears, an example of the original product to be replaced.

4.5.2.2. *Regulatory aspects and sustainability*

No sustainable references of jelly or candy comparable to this product have been found.

Regarding the regulatory aspects, all those listed in section 4.1.1 apply to this product. For gummy bears, it is not clear if they would need to be considered a food or a food supplement, as they are intended to supply most of the B-group vitamins in levels close to the recommended daily intake. Food supplements are legally considered as food, and so, in general, the same regulation as general food applies. However, for the vitamin and mineral supplementation, a specific directive applies for the supplements: Directive 2002/46/EC. This directive also sets out special labelling requirements for food supplements. As for vitamin and minerals fortified foods, there is still not a regulation regarding the maximum allowed levels, but this topic is under discussion in the EC and thus, it may be regulated in the upcoming years.

4.5.2.3. *Essential attributes for manufacturing*

The serving size is expected to be of 2 gummy bears, each of them of 2,5 g. So, the serving will be of 5 g. The production cost is expected to be less than 0.2€ per serving.

Regarding the manufacturing process, all the ingredients can be either dried or in liquid format. It will be especially necessary to look for the right incorporation of the oils in the gelatine. To achieve so, the right choice of the gum mixture will be crucial, as depending on which they are used, the cooling process might be faster or sluggish. In order to achieve a good incorporation of oils, a fast-cooling process will be desirable.

4.5.2.4. *Quantities to be produced and specific amounts of required ingredients*

As part of the INNOAQUA project, the vegan gummy bears will be developed following different steps. The first formulation tests will be performed in kitchen-scale to obtain the first prototype. This prototype will be characterized and tested, and if it achieves a good performance, it will be later scaled-up to industrial level and produced at larger amounts with the aim to be introduced into the market of some EU countries.

The task T4.3 “Formulation and validation of food products” starts at M20. By November of 2025 (M30), it is expected that the kitchen prototypes will have been developed. A total of 300g of gummy bears is expected to be produced during this period. However, the necessary ingredients will be needed by M20 to be able to perform the necessary tests and formulate the prototypes. For the accelerated shelf-life studies, which will be performed at the end of 2025, a total of 100g of the product will be necessary. Later (during 2027; M44-48), and after industrial scaling up (if it applies), the sensory tests and consumer acceptance tests will be performed. For the first, approximately 300g will be necessary, while for the consumer acceptance test, 150g of gummy bears will be produced.

4.5.2.5. *Methods and criteria for assessing performance*

To assess the performance of the product kitchen prototype, and later the industrial prototype, several tests will be necessary:

- Accelerated shelf-life test: it will be necessary to establish the amount of time that the product can be stored without losing its properties and the adequacy to be consumed.

To do so, accelerated shelf-life test will be performed: first in the kitchen- prototype

and later the final industrial prototype. It is expected for the product to have a shelf-life of at least **6 months**.

- Consumer acceptance: information regarding the consumer acceptance of the products will be provided by WP1. However, as part of the assessment of the product quality, an additional sensory (taste, colour, texture, smell...) consumer acceptance evaluation will be performed as part of task T4.3. This evaluation will be performed at the end of the industrial developing stage, with a panel of at least 40 consumers.

In addition of the general consumer acceptance parameters described in section 4.1.2, the following parameters have been identified as especially relevant for the vegan gummy bears: **Good flavour (sweet, fruity taste), good sensory experience: chewy, tender, good tactile eating experience, good vitamin profile and intake.**

- Sensory tests: the sensory evaluation of the prototype will be performed by an expert panel, consisting of a minimum of 8 taster experts trained for this purpose. These evaluations will be conducted at the end of the industrial stage.
- General characterization, including the assessing of iodine and heavy metals content, to make sure that they are according to the regulations, as well as biological safety tests.

4.5.3. Energy bars

4.5.3.1. *General description*

The next vegan snack that we will develop is an energy bar. This will be a sweet protein rich snack which may contain cereals nuts and dried fruits. It will also be enriched with vitamins.

The consumer target for this product are sporty people looking for some energy before or after a high intensity activity. In this sense, the product intends to provide at least 300 kcal /100g. To achieve so, it will contain a high amount of protein (20-30%), which will come from the INNOAQUA microalgae, combine with carbohydrates coming from nuts and cereals. To provide a more interesting nutritional profile, the bars will be also enriched with microalgae hydrophilic vitamins (less than 0.1%) and microalgae peptides and amino acids (less than 5%).

As the protein will come from microalgae, we expect the product to have an excellent amino acid profile.

The product will have the shape of a bar, with a thick or crunchy consistency. A thin layer of chocolate may be used to cover the product and preserve the interior. Dried fruits may be added as sweeteners and to provide a pleasant flavour. Some sugar may also be incorporated, even it will try to be minimized, and the addition of artificial sweeteners is intended to be avoided.



Figure 14. Image of Energy bar, an example of the original product to be replaced. Source : PowerCrunch

4.5.3.2. *Regulatory aspects and sustainability*

Even we could not find EPD references for energy bars, it may be useful to compare the current product with chocolate and nuts granola (30), with a carbon footprint of 1.96 kg CO₂ eq, or a chocolate coated biscuit (36), with a carbon footprint of 4.76 kg CO₂ eq.

Regarding the regulatory aspects, all those listed in section 4.1.1 apply to this product.

4.5.3.3. *Essential attributes for manufacturing*

The serving size is expected to be a single bar of around 50g. The production cost is expected to be less than 1€ per serving.

The product will need to have a compact texture. To achieve so, the ingredients will need to be provided in dried format. In addition, and as a strategy to extend the shelf-life of the product, the bars will probably have a chocolate coating.

4.5.3.4. *Quantities to be produced and specific amounts of required ingredients*

As part of the INNOAQUA project, the vegan energy bars will be developed following different steps. The first formulation tests will be performed in kitchen-scale to obtain the first prototype. This prototype will be characterized and tested, and if it achieves a good performance, it will be later scaled-up to industrial level and produced at larger amounts with the aim to be introduced into the market of some EU countries.

The task T4.3 “Formulation and validation of food products” starts at M20. By November of 2025 (M30), it is expected that the kitchen prototypes will have been developed. A total of 1 kg of energy bars is expected to be produced during this period. However, the necessary ingredients will be needed by M20 to be able to perform the necessary tests and formulate the prototypes. For the accelerated shelf-life studies, which will be performed at the end of 2025, a total of 500g of the product will be necessary. Later (during 2027; M44-48), and after industrial scaling up (if it applies), the sensory tests and consumer acceptance tests will be performed. For the first, approximately 56 servings are necessary, while for the later, the minimum amount will be 200 servings. Thus, a total of 15kg will be produced to supply for both tests, while a minimum industrial production of 30 kg is expected.

4.5.3.5. *Methods and criteria for assessing performance*

To assess the performance of the product kitchen prototype, and later the industrial prototype, several tests will be necessary:

- Accelerated shelf-life test: it will be necessary to establish the amount of time that the product can be stored without losing its properties and the adequacy to be consumed. To do so, accelerated shelf-life test will be performed: first in the kitchen- prototype and later the final industrial prototype. It is expected for the product to have a shelf-life of at least **6 months**.
- Consumer acceptance: information regarding the consumer acceptance of the products will be provided by WP1. However, as part of the assessment of the product quality, an additional sensory (taste, colour, texture, smell...) consumer acceptance evaluation will be performed as part of task T4.3. This evaluation will be performed at the end of the industrial developing stage, with a panel of at least 40 consumers.

In addition of the general consumer acceptance parameters described in section 4.1.2, the following parameters have been identified as especially relevant for the energy bars: **High energy, high protein, good flavour, low sugars, no palm oil, clean label.**

- Sensory tests: the sensory evaluation of the prototype will be performed by an expert panel, consisting of a minimum of 8 taster experts trained for this purpose. These evaluations will be conducted at the end of the industrial stage.
- General characterization, including the assessing of iodine and heavy metals content, to make sure that they are according to the regulations, as well as biological safety tests.

4.5.4. Energy balls

4.5.4.1. *General description*

The last of the products in the vegan snacks category is the energy ball. An energy ball is a sweet treat made of dates, fruits and nuts intending to provide a high energy intake and a satiating effect. It is often perceived as a healthy snack, filled with healthy ingredients and with a good nutritional profile. Thus, it is commonly enriched with protein and vitamins and sweetened with fruits. The main difference of energy balls compared to energy bars, besides the shape, is that energy balls have a higher content of carbohydrates and a lower content of proteins compared to the energy bars. Our version of the product, which development will be led by ALGEMY, will be prepared using INNOAQUA's microalgae ingredients.

The consumer target for this product are sporty people looking for some energy before or after a high intensity activity. In this sense, the product intends to provide at least 350 kcal /100g. To achieve so, it will contain a high amount of carbohydrates, coming from nuts and fruits, and protein (15-25%), which will come from the INNOAQUA microalgae. To provide a more interesting nutritional profile, the energy balls will be also enriched with microalgae hydrophilic vitamins (less than 0.1%) and microalgae peptides and amino acids (less than 5%). As the protein will come from microalgae, we expect the product to have an excellent amino acid profile.

The product will have the shape of a ball, with a compact and sticky texture. A thin layer of chocolate may be used to coat the product and preserve the interior. Fruit, and specially dates, will be added as sweeteners and carbohydrate suppliers, and to provide a pleasant flavour. Some sugar may also be incorporated, even it will try to be minimized, and the addition of artificial sweeteners is intended to be avoided.



t 15. Image of Energy ball, an example of the original product to be replaced. Source: KoRo.

4.5.4.2. *Regulatory aspects and sustainability*

Even we could not find EPD references for energy balls, it may be useful to compare the current product with chocolate and nuts granola (30), with a carbon footprint of 1.96 kg CO₂ eq or a chocolate coated biscuit (36), with a carbon footprint of 4.76 kg CO₂ eq.

Regarding the regulatory aspects, all those listed in section 4.1.1 apply to this product.

4.5.4.3. *Essential attributes for manufacturing*

The serving size is expected to be a single ball of 30-40g, probably packed individually. The production cost is expected to be less than 1€ per serving.

The product will need to have a compact texture. To achieve so, the INNOAQUA ingredients will need to be provided in dried format. In addition, and as a strategy to extend the shelf-life of the product, the balls will probably have a chocolate coating.

4.5.4.4. *Quantities to be produced and specific amounts of required ingredients*

As part of the INNOAQUA project, the vegan energy balls will be developed following different steps. The first formulation tests will be performed in kitchen-scale to obtain the first prototype. This prototype will be characterized and tested, and if it achieves a good performance, it will be later scaled-up to industrial level and produced at larger amounts with the aim to be introduced into the market of some EU countries.

The task T4.3 “Formulation and validation of food products” starts at M20. By November of 2025 (M30), it is expected that the kitchen prototypes will have been developed. A total of 1 kg of energy balls is expected to be produced during this period. However, the necessary ingredients will be needed by M20 to be able to perform the necessary tests and formulate the prototypes. For the accelerated shelf-life studies, which will be performed at the end of 2025, a total of 500g of the product will be necessary. Later (during 2027; M44-48), and after industrial scaling up (if it applies), the sensory tests and consumer acceptance tests will be performed. For the first, approximately 56 servings are necessary, while for the later, the minimum amount will be 200 servings. Thus, a total of 10kg will be produced to supply for both tests, while a minimum industrial production of 20 kg is expected.

4.5.4.5. *Methods and criteria for assessing performance*

To assess the performance of the product kitchen prototype, and later the industrial prototype, several tests will be necessary:

- Accelerated shelf-life test: it will be necessary to establish the amount of time that the product can be stored without losing its properties and the adequacy to be consumed. To do so, accelerated shelf-life test will be performed: first in the kitchen- prototype and later the final industrial prototype. It is expected for the product to have a shelf-life of at least **6 months**.
- Consumer acceptance: information regarding the consumer acceptance of the products will be provided by WP1. However, as part of the assessment of the product quality, an additional sensory (taste, colour, texture, smell...) consumer acceptance evaluation will be performed as part of task T4.3. This evaluation will be performed at the end of the industrial developing stage, with a panel of at least 40 consumers.

In addition of the general consumer acceptance parameters described in section 4.1.2, the following parameters have been identified as especially relevant for the energy balls: **High energy, good flavour (fruity, chocolate...), low sugars, no palm oil, clean label.**

- Sensory tests: the sensory evaluation of the prototypes will be performed by an expert panel, consisting of a minimum of 8 taster experts trained for this purpose. These evaluations will be conducted at the end of the industrial stage.
- General characterization, including the assessing of iodine and heavy metals content, to make sure that they are according to the regulations, as well as biological safety tests.

4.6. Other vegan food

A last category of vegan products is included, in where we can fit all the products that do not belong in any of the other categories. Two products are included in this category: the vegan shots and the vegan spreads.

4.6.1. Shots

4.6.1.1. *General description*

The first product on this category is a vegan shot, a beverage with a liquid consistency and enriched with vitamins and proteins. Vegan shots are perceived as very healthy drinks that can provide a lot of energy for the day and supply a considerable amount of vitamins. They are sweet, with a fruity flavour, as the main component is usually fruit juice.

In this case, the product will be vegan and will incorporate the INNOAQUA microalgae hydrophilic vitamins (less than 1% of the total weight), as well as microalgae PUFA's and carotenoid rich oils (less than 1%). This will make the product very rich in B vitamins, as well as omega-3. It is also expected to be rich in minerals, such as zinc, as the microalgae vitamins ingredient will also have a high mineral content.

Regarding the proteins, it is expected for the product to have a protein content higher than 40%, but the protein source will not be necessary the INNOAQUA's algae.

VIVA MARIS will be in charge of the development of this product.



Figure 16. Image of the expected appearance of the product. Source: VIVA MARIS.

4.6.1.2. *Regulatory aspects and sustainability*

Even we could not find EPD references for vegan shots, it may be useful to compare the current product with fruit juices (37; 38), with carbon footprints of 0.38 and 0.45 kg CO₂ eq., respectively, or a plant-based milk enriched with vitamins (39), with a carbon footprint of 0.943 kg CO₂ eq.

Regarding the regulatory aspects, all those listed in section 4.1.1 apply to this product.

4.6.1.3. *Essential attributes for manufacturing*

The serving size of this product will be 100 mL of liquid served in individual glass bottles. The production cost is expected to be lower than 0.9€ / bottle.

As the final product is liquid, all the ingredients should be preferable provided in a liquid format, or, if they are powdered, they may have a high level of water solubility. Regarding the final product properties, it is important for it to have a good taste and smell, and to not leave an unpleasant aftertaste.

4.6.1.4. *Quantities to be produced and specific amounts of required ingredients*

As part of the INNOAQUA project, the vegan shots will be developed following different steps. The first formulation tests will be performed in kitchen-scale to obtain the first prototype. This prototype will be characterized and tested, and if it achieves a good performance, it will be later scaled-up to industrial level and produced at larger amounts with the aim to be introduced into the market of some EU countries.

The task T4.3 “Formulation and validation of food products” starts at M20. By November of 2025 (M30), it is expected that the kitchen prototypes will have been developed. A total of 1L of vegan shots is expected to be produced during this period. However, the necessary ingredients will be needed by M20 to be able to perform the necessary tests and formulate the prototypes. For the accelerated shelf-life studies, which will be performed at the end of 2025, a total of 500mL of the product will be necessary. Later (during 2027; M44-48), and after industrial scaling up (if it applies), the sensory tests and consumer acceptance tests will be performed. For the first, approximately 500mL of the product will be necessary, while ca. 3 L will be needed for the later.

4.6.1.5. *Methods and criteria for assessing performance*

To assess the performance of the product kitchen prototype, and later the industrial prototype, several tests will be necessary:

- Accelerated shelf-life test: it will be necessary to establish the amount of time that the product can be stored without losing its properties and the adequacy to be consumed. To do so, accelerated shelf-life test will be performed: first in the kitchen- prototype and later the final industrial prototype. It is expected for the product to have a shelf-life of at least **24 months**.
- Consumer acceptance: information regarding the consumer acceptance of the products will be provided by WP1. However, as part of the assessment of the product quality, an additional sensory (taste, colour, texture, smell...) consumer acceptance evaluation will be performed as part of task T4.3. This evaluation will be performed at the end of the industrial developing stage, with a panel of at least 40 consumers.

In addition of the general consumer acceptance parameters described in section 4.1.2, the following parameters have been identified as especially relevant for the vegan shots: **Liquid structure, no aftertaste, high protein, high in vitamins and zinc, good taste and smell.**

- Sensory tests: the sensory evaluation of the prototype will be performed by an expert panel, consisting of a minimum of 8 taster experts trained for this purpose. These evaluations will be conducted at the end of the industrial stage.
- General characterization, including the assessing of iodine and heavy metals content, to make sure that they are according to the regulations, as well as biological safety tests.

4.6.2. Spreads

4.6.2.1. *General description*

The last of the vegan prototypes is a spread. It is a product with soft consistency intended to be spread onto bread. It will have a vegetable base and will be enriched with vitamins and proteins to have a high energetic content. VIVA MARIS will lead the development of this product.

The consistency of the product will be soft and smooth to allow for the consumer to spread the product easily. Regarding the composition, it will contain more than a 40% of its weight in proteins, which will come both from microalgae (up to 30%) and macroalgae (up to 30%). In addition, it will contain microalgae PUFA's and carotenoid rich oil (up to 1%), that will provide omega-3, and will also be enriched with B-vitamins.



Figure 17. Image of the expected appearance of the product. Source : VIVA MARIS.



4.6.2.2. Regulatory aspects and sustainability

Even we could not find EPD references for bread-spreads, it may be useful to compare the current product with a vegan pesto sauce, with a carbon footprint of 3.61 kg CO₂ eq (40).

Regarding the regulatory aspects, all those listed in section 4.1.1 apply to this product.

4.6.2.3. Essential attributes for manufacturing

The format of this product will be 180 g of spread, packed in a glass container. The production cost is expected to be lower than 1.70€ / unit.

As the final product is solid, the proteins and vitamins should be provided in a dried format. The PUFA's and carotenoids rich oil will be a liquid. As the final product should avoid green and brownish colours for consumers to accept it, it is important for the ingredients (and specially the proteins) to be as neutral in colour as possible.

4.6.2.4. Quantities to be produced and specific amounts of required ingredients

As part of the INNOAQUA project, the vegan spreads will be developed following different steps. The first formulation tests will be performed in kitchen-scale to obtain the first prototype. This prototype will be characterized and tested, and if it achieves a good performance, it will be later scaled-up to industrial level and produced at larger amounts with the aim to be introduced into the market of some EU countries.

Task T4.3 "Formulation and validation of food products" starts at M20. By November of 2025 (M30), it is expected that the kitchen prototypes will have been developed. A total of 5 kg of vegan spread is expected to be produced during this period. However, the necessary ingredients will be needed by M20 to be able to perform the necessary tests and formulate the prototypes. For the accelerated shelf-life studies, which will be performed at the end of 2025, a total of 500 g of the product will be necessary. Later on (during 2027; M44-48), and after industrial scaling up (if it applies), the sensory tests and consumer acceptance tests will be performed. For the first, approximately 500 g of the product will be necessary, while ca. 1 kg will be needed for the later.

4.6.2.5. *Methods and criteria for assessing performance*

To assess the performance of the product kitchen prototype, and later the industrial prototype, several tests will be necessary:

- Accelerated shelf-life test: it will be necessary to establish the amount of time that the product can be stored without losing its properties and the adequacy to be consumed. To do so, accelerated shelf-life test will be performed: first in the kitchen- prototype and later the final industrial prototype. It is expected for the product to have a shelf-life of at least **6 months**.
- Consumer acceptance: information regarding the consumer acceptance of the products will be provided by WP1. However, as part of the assessment of the product quality, an additional sensory (taste, colour, texture, smell...) consumer acceptance evaluation will be performed as part of task T4.3. This evaluation will be performed at the end of the industrial developing stage, with a panel of at least 40 consumers. In addition of the general consumer acceptance parameters described in section 4.1.2, the following parameters have been identified as especially relevant for the vegan bread spreads: **soft spreadable structure, no aftertaste, low in salt, no green or brown colour, high protein content, good smell and taste**.
- Sensory tests: the sensory evaluation of the prototype will be performed by an expert panel, consisting of a minimum of 8 taster experts trained for this purpose. These evaluations will be conducted at the end of the industrial stage.
- General characterization, including the assessing of iodine and heavy metals content, to make sure that they are according to the regulations, as well as biological safety tests.

4.7. Hybrid products

Hybrid products are those that combine animal and plant-based ingredients. This mixture allows for us to take advantage from both ingredient; namely, being a highly sustainable, healthy and animal cruelty free product while preserving the flavour and texture of the animal



products. Thus, these products become a good option to reduce animal ingredients ingestion without completely giving them up.

As part of the INNOAQUA project, a group of hybrid products will be developed, which will include sustainable fish and algae ingredients, as the fish protein will be replaced by protein, vitamins and omega-3 obtained from algae and from fish by-products (fish protein hydrolysates; FPH). Most of the hybrid products that will be developed have their vegan brother version, and so, they will consist of a modification of the recipe to replace part of the algae ingredients with those coming from the fish by-products.

4.7.1. Fish bar

4.7.1.1. *General description*

The first hybrid fish-algae product is a fish filet that will include sustainable protein coming from algae and fish by-products. The idea is to reduce the amount of fish (in this case, fish by-products) in the final product by mixing it with microalgae protein (ca. 5% of the product weight) and macroalgae protein (a maximum of 10%), while obtaining a texture, flavour and juiciness like that of fish. Fish protein hydrolysates obtained from fish by-products (salmon heads and skins or non-compliant surimi sticks) will be incorporated in an amount of around 1%, which will complement the amino acid profile of algae. Macroalgae agar (less than 1%) and the macroalgae residual fraction (less than 2%) may be incorporated to improve the product texture.

The intended nutritional attributes of the product are around a 15% of protein, between a 2 and a 4 % of fat a maximum of 2% of carbohydrates and around a 0.3% of omega-3 (EPA+DHA). The moisture content of the product is expected to be between 78 and 82%.

PESCANOVA will be in charge of the development of this prototype.

4.7.1.2. *Regulatory aspects and sustainability*

Products such as Atlantic salmon (25), with a carbon footprint of 4.74 kg CO₂ eq., king salmon (26), with a carbon footprint of 13.3 kg CO₂ eq., or other fish products (27), with a carbon footprint of 4.999 kg CO₂ eq., can be used as sustainability references.



Regarding the regulatory aspects, all those listed in section 4.1.1 apply to this product.

4.7.1.3. Essential attributes for manufacturing

The serving size will be a portion of 80-100g. The expected production cost per serving is between 3 and 4€.

To achieve the desired texture for the product, the protein will need to be dried and powdered, or a wet extrusion process may be necessary. The omega-3 fatty acids may be either dried and powdered or in a liquid format.

It is also important for the dough to be white, to resemble the original fish fillets, or if it is not possible, we will look for a pink colour to resemble salmon fillets. The water holding capacity of the dough and final product is also important, and it should be stable in freeze.

4.7.1.4. Quantities to be produced and specific amounts of required ingredients

As part of the INNOAQUA project, the hybrid bars will be developed following different steps. The first formulation tests will be performed in kitchen-scale to obtain the first prototype. This prototype will be characterized and tested, and if it achieves a good performance, it will be later scaled-up to industrial level and produced at larger amounts with the aim to be introduced into the market of some EU countries.

The task T4.3 “Formulation and validation of food products” starts at M20. By November of 2025 (M30), it is expected that the kitchen prototypes will have been developed. A total of 5kg of hybrid bars are expected to be produced during this period. However, the necessary ingredients will be needed by M20 to be able to perform the necessary tests and formulate the prototypes. For the accelerated shelf-life studies, which will be performed at the end of 2025, a total of 2kg of the product will be necessary. Later (during 2027; M44-48), and after industrial scaling up (if it applies), the sensory tests and consumer acceptance tests will be performed. For the first, approximately 56 servings are necessary, while for the later, the minimum amount will be 200 servings. Thus, a total of 22kg will be produced to supply for both tests, while a total industrial production of 100-200 kg is planned.

4.7.1.5. *Methods and criteria for assessing performance.*

To assess the performance of the product kitchen prototype, and later the industrial prototype, several tests will be necessary:

- Accelerated shelf-life test: it will be necessary to establish the amount of time that the product can be stored without losing its properties and the adequacy to be consumed. To do so, accelerated shelf-life test will be performed: first in the kitchen- prototype and later on the final industrial prototype. It is expected for the product to have a shelf-life of at least **18 months frozen at -18°C**.
- Consumer acceptance: information regarding the consumer acceptance of the products will be provided by WP1. However, as part of the assessment of the product quality, an additional sensory (taste, colour, texture, smell...) consumer acceptance evaluation will be performed as part of task T4.3. This evaluation will be performed at the end of the industrial developing stage, with a panel of at least 40 consumers. In addition of the general consumer acceptance parameters described in section 4.1.2, the following parameters have been identified as especially relevant for the hybrid fish bars: **texture like fish fillet, juicy, sea flavour. White colour. If protein is dark, we could maybe try to produce salmon analogue.**
- Sensory tests: the sensory evaluation of the prototype will be performed by an expert panel, consisting of a minimum of 8 taster experts trained for this purpose. An additional product assessment panel (at least 10 members) will also validate the product. These evaluations will be conducted at the end of the industrial stage.
- General characterization, including the assessing of iodine and heavy metals content, to make sure that they are according to the regulations, as well as biological safety tests.

4.7.2. Nuggets

4.7.2.1. *General description*

The second hybrid product to be developed are the hybrid algae-fish nuggets. This product will consist of small pieces of texturized protein that will be breaded or battered and finally deep-fried or baked. They will resemble the chicken nuggets largely available in the market.

The product will need to have a high protein content (higher than 20%), as well as a vitamin enrichment. In addition, the product is expected to have a good colour (golden brown), juiciness and a fibrous texture resembling meat. To achieve these requirements, some of the ingredients produced during the INNOAQUA project will be employed, namely microalgae and macroalgae protein (up to a 30% each), fish protein hydrolysates obtained from fish by-products (up to a 5%) and it may also contain microalgae PUFA's (up to 1%). Depending on the quality of the INNOAQUA protein (specially in terms of colour), protein obtained from other vegan sources (e.g., soy, pea, chickpea) may be also included in the blend.

The development of this prototype will be led by LEITAT.

4.7.2.2. *Regulatory aspects and sustainability*

For the sustainability requirements definition, a reference consisting in chicken breast, with a carbon footprint of 5.96 kg CO₂ eq. has been found (24). Other more general useful references could be those of veal meat (22; 23), with carbon footprints of 29.8 (fresh format) and 27 kg CO₂ eq. (including cooking), respectively.

Regarding the regulatory aspects, all those listed in section 4.1.1 apply to this product.

4.7.2.3. *Essential attributes for manufacturing*

The serving size for this product is estimated to be of 100g, which will correspond to six pieces of nuggets. The expected production cost for the serving is of around 3€.

To achieve a product resembling the original meat nuggets, it is very important for the protein to be texturized, to obtain a fibrous texture. A dry extrusion process will most probably be needed. In addition, the product is expected to be juicy and with a soft and non-sticky texture.

The inside colour of the final product will also be relevant, to have a good consumer acceptance. Green or brownish colours, typical from algae biomass need to be avoided.

As already stated in the previous section, this product will be developed by the partner LEITAT. However, LEITAT only have the capability to produce food products up to a kitchen scale. Thus, either this product won't be able to be selected for the industrial scale production, or a third party will be necessary to scale the production up to an industrial stage.

4.7.2.4. Quantities to be produced and specific amounts of required ingredients

As part of the INNOAQUA project, the hybrid nuggets will be developed following different steps. The first formulation tests will be performed in kitchen-scale to obtain the first prototype. This prototype will be characterized and tested, and if it achieves a good performance, its later scale-up production to industrial level may be consider, even the partner in charge of this product (LEITAT) does not have the capabilities to do so.

Task T4.3 "Formulation and validation of food products" starts at M20. By November of 2025 (M30), it is expected that the kitchen prototypes will have been developed. A total of 2kg of hybrid nuggets are expected to be produced during this period. However, the necessary ingredients will be needed by M20 to be able to perform the necessary tests and formulate the prototypes. For the accelerated shelf-life studies, which will be performed at the end of 2025, a total of 300g of the product will be necessary. Later (during 2027; M44-48), and after industrial scaling up (if it applies), the sensory tests and consumer acceptance tests would be performed. For the first, approximately 300g of the product would be necessary, while ca. 5 kg would be needed for the later.

4.7.2.5. Methods and criteria for assessing performance

To assess the performance of the product kitchen prototype, and later the industrial prototype (if scaled-up), several tests will be necessary:

- Accelerated shelf-life test: it will be necessary to establish the amount of time that the product can be stored without losing its properties and the adequacy to be consumed. To do so, accelerated shelf-life test will be performed: first in the kitchen- prototype and later, on the final industrial prototype (if produced). It is expected for the product to have a shelf-life of at least **six months under frozen conditions**.

- Consumer acceptance: information regarding the consumer acceptance of the products will be provided by WP1. However, as part of the assessment of the product quality, an additional sensory (taste, colour, texture, smell...) consumer acceptance evaluation will be performed as part of task T4.3, **only if this product is scaled-up to industrial level**. This evaluation would be performed at the end of the industrial developing stage, with a panel of at least 40 consumers.

In addition of the general consumer acceptance parameters described in section 4.1.2, the following parameters have been identified as especially relevant for the hybrid nuggets: **Good flavour, crunchiness, juiciness, no aftertaste, high protein content, fibrous texture but soft at bite**.

- Sensory tests: the sensory evaluation of the prototype will be performed by an expert panel, consisting of a minimum of 8 taster experts trained for this purpose. This evaluation will be conducted at the end of the industrial stage, **only if the product is scaled up to industrial level**.
- General characterization, including the nutritional composition, assessing of iodine and heavy metals content, to make sure that they are according to the regulations, as well as biological safety tests.

4.7.3. Sausages

4.7.3.1. *General description*

The goal is to produce hybrid fish-algae sausages using the protein and other ingredients produced as part of the INNOAQUA project, which will resemble meat sausages. The INNOAQUA ingredients will need to provide the product with a solid consistency and a good vitamin and amino acid profile. This product will be developed with the lead of the partner VIVA MARIS.

The final product is expected to have a nice texture, resembling the meat sausages, and with a protein content of at least 40% of the product weight. The protein blend will include protein obtained from macroalgae (up to 30%), microalgae (up to 30%) and fish by-products (FPH's; up to 5%). The product will also be enriched with the hydrophilic B vitamins obtained from microalgae, as well as with poly-unsaturated fatty acids obtained from microalgae (a

maximum of 1% of the total product weight), which will provide the final product with an excellent nutritional profile and a high energy content.

4.7.3.2. Regulatory aspects and sustainability

For the sustainability requirements definition, a reference consisting in pork sausages has been found (20) (with a carbon footprint of 3.7 kg CO₂ eq., including the cooking), as well as a LCA document about plant-based sausages (21) from Impossible Foods Inc, with a carbon footprint of 2.09 kg CO₂ eq. Other more general useful references could be those of veal meat (22; 23), with carbon footprints of 29.8 kg CO₂ eq. (fresh format) and 27 kg CO₂ eq. (including cooking), respectively.

Regarding the regulatory aspects, all those listed in section 4.1.1 apply to this product.

4.7.3.3. Essential attributes for manufacturing

The sausages will weigh around 60 g each and will be served in packages of 4 units. Thus, 240g per serving. It is expected that the manufacturing cost of each serving will be around 1.80€.

For its manufacturing, the texture and colour will be essential. It is important that the end product does not have a strong green or brownish colour, as it would disincentivize consumer acceptance. The texture is also important, as it needs to be solid and resembling that of meat-sausages. Thus, a texturization of the protein could be necessary.

4.7.3.4. Quantities to be produced and specific amounts of required ingredients

As part of the INNOAQUA project, the hybrid sausage will be developed following different steps. The first formulation tests will be performed in kitchen-scale to obtain the first prototype. This prototype will be characterized and tested, and if it achieves a good performance, it will be later scaled-up to industrial level and produced at larger amounts with the aim to be introduced into the market of some EU countries.

The task T4.3 “Formulation and validation of food products” starts at M20. By November of 2025 (M30), it is expected that the kitchen prototypes will have been developed. A total of 5kg of hybrid sausages are expected to be produced during this period. However, the necessary ingredients will be needed by M20 to be able to perform the necessary tests and formulate the prototypes. For the accelerated shelf-life studies, which will be performed at

the end of 2025, a total of 500g of the product will be necessary. Later (during 2027; M44-48), and after industrial scaling up (if it applies), the sensory tests and consumer acceptance tests will be performed. For the first, approximately 500g of the product will be necessary, while ca. 3 kg will be needed for the later.

4.7.3.5. *Methods and criteria for assessing performance*

To assess the performance of the product kitchen prototype, and later the industrial prototype (if scaled-up), several tests will be necessary:

- Accelerated shelf-life test: it will be necessary to establish the amount of time that the product can be stored without losing its properties and the adequacy to be consumed. To do so, accelerated shelf-life test will be performed: first in the kitchen- prototype and later on the final industrial prototype. It is expected for the product to have a shelf-life of at least **six months**.
- Consumer acceptance: information regarding the consumer acceptance of the products will be provided by WP1. However, as part of the assessment of the product quality, an additional sensory (taste, colour, texture, smell...) consumer acceptance evaluation will be performed as part of task T4.3. This evaluation will be performed at the end of the industrial developing stage, with a panel of at least 40 consumers.

In addition of the general consumer acceptance parameters described in section 4.1.2, the following parameters have been identified as especially relevant for the hybrid sausages: **solid structure, no aftertaste, low salt content, no green or brown colour, high protein content, good bite and good smell**.

- Sensory tests: the sensory evaluation of the prototype will be performed by an expert panel, consisting of a minimum of 8 taster experts trained for this purpose. This evaluation will be conducted at the end of the industrial stage.
- General characterization, including the assessing of iodine and heavy metals content, to make sure that they are according to the regulations, as well as biological safety tests.

4.7.4. Bread spreads

4.7.4.1. *General description*

The last of the hybrid algae-fish prototypes is a spread. It is a product with soft consistency intended to be spread onto bread. It will have a vegetable base and will be enriched with vitamins and proteins to have a high energetic content. VIVA MARIS will lead the development of this product.

The consistency of the product will be soft and smooth, to allow for the consumer to spread the product easily. Regarding the composition, it will contain more than a 40% of its weight in proteins, which will come both from microalgae (up to 30%), macroalgae (up to 30%) and fish by-products (FPH's; up to 5%). In addition, it will contain microalgae PUFA's and carotenoid rich oil (up to 1%), which will provide omega-3, and will also be enriched with B-vitamins.

4.7.4.2. *Regulatory aspects and sustainability*

Even we could not find EPD references for bread-spreads, it may be useful to compare the current product with a vegan pesto sauce (40), with a carbon footprint of 3.61 kg CO₂ eq.

Regarding the regulatory aspects, all those listed in section 4.1.1 apply to this product.

4.7.4.3. *Essential attributes for manufacturing*

The format of this product will be 180 g of spread, packed in a glass container. The production cost is expected to be lower than 1.70€ / unit.

As the final product is solid, the proteins and vitamins should be provided in a dried format. The PUFA's and carotenoids rich oil will be a liquid. As the final product should avoid green and brownish colours for consumers to accept it, it is important for the ingredients (and specially the proteins) to be as neutral in colour as possible.

4.7.4.4. *Quantities to be produced and specific amounts of required ingredients*

As part of the INNOAQUA project, the hybrid spreads will be developed following different steps. The first formulation tests will be performed in kitchen-scale to obtain the first prototype. This prototype will be characterized and tested, and if it achieves a good performance, it will be later scaled-up to industrial level and produced at larger amounts with the aim to be introduced into the market of some EU countries.

The task T4.3 “Formulation and validation of food products” starts at M20. By November of 2025 (M30), it is expected that the kitchen prototypes will have been developed. A total of 5 kg of hybrid spread is expected to be produced during this period. However, the necessary ingredients will be needed by M20 to be able to perform the necessary tests and formulate the prototypes. For the accelerated shelf-life studies, which will be performed at the end of 2025, a total of 500 g of the product will be necessary. Later (during 2027; M44-48), and after industrial scaling up (if it applies), the sensory tests and consumer acceptance tests will be performed. For the first, approximately 500 g of the product will be necessary, while ca. 1 kg will be needed for the later.

4.7.4.5. Methods and criteria for assessing performance

To assess the performance of the product kitchen prototype, and later the industrial prototype, several tests will be necessary:

- Accelerated shelf-life test: it will be necessary to establish the amount of time that the product can be stored without losing its properties and the adequacy to be consumed. To do so, accelerated shelf-life test will be performed: first in the kitchen- prototype and later the final industrial prototype. It is expected for the product to have a shelf-life of at least **6 months**.
- Consumer acceptance: information regarding the consumer acceptance of the products will be provided by WP1. However, as part of the assessment of the product quality, an additional sensory (taste, colour, texture, smell...) consumer acceptance evaluation will be performed as part of task T4.3. This evaluation will be performed at the end of the industrial developing stage, with a panel of at least 40 consumers. In addition of the general consumer acceptance parameters described in section 4.1.2, the following parameters have been identified as especially relevant for the hybrid bread spreads: **soft spreadable structure, no aftertaste, low in salt, no green or brown colour, high protein content, good smell and taste**.
- Sensory tests: the sensory evaluation of the prototype will be performed by an expert panel, consisting of a minimum of 8 taster experts trained for this purpose. These evaluations will be conducted at the end of the industrial stage.

- General characterization, including the assessing of iodine and heavy metals content, to make sure that they are according to the regulations, as well as biological safety tests.

4.8. Biodegradable packaging

With the residual fractions of microalgae and macroalgae, a biobased plastic resin will be produced, which will later be used to develop different biodegradable packaging solutions. The different packaging units will be evaluated on their own, but also as containers for some of the seafood products developed during the INNOAQUA project.

4.8.1. Lidding films

4.8.1.1. *General description*

The first packaging solution will be a lidding film made from conventional plastic and residual algae fraction which will be used to close the trays for the food products.

The final characteristics and production process of the lidding films will be adapted depending on the composition and properties of the residual algae fraction, but the residual algae fraction will suppose 25 to 50% of the composition of the lidding films. In addition, other conventional plastic resins such as PE, PP, HDPE or LDPE may be used. LDPE is a recyclable material and is commonly used for this purpose, and so, the utilization of this resin will be the main field to be studied. In addition, the possibility to make a compostable product will be studied.

The film will need to have 470mm of width and should be sealable for testing assembly with the tray.

The development of the lidding films will be part of T4.4 and will be led by ERANOVA. ERANOVA is a bioplastic compounder and not a bioplastic object producer, meaning that they are producing the bioresins, but are not able to shape them into the final plastic products. Thus, a plastic transformer external from the INNOAQUA project will be needed to shape the final lids.

The lids, together with the trays will be used to assemble a packaging solution which will be tested with some of the INNOAQUA seafood products. The selected products for this test are the vegan fish filets and vegan surimi.

4.8.1.2. Regulatory aspects and sustainability

For the sustainability requirements definition, products such as plastic films (41; 42), with carbon footprints of 0.58 and 0.62 kg CO₂ eq., respectively, or polyethylene foam films (43), with a carbon footprint of 0.072 kg CO₂ eq. can serve as references.

For the packaging solutions, several regulations will apply, as these containers will be in contact with food products. The most relevant ones are:

- Good manufacturing practice for materials and articles intended to come into contact with food. Regulation (EC) 1935/2004;
- Plastic materials and articles intended to come into contact with food. Commission Regulation (EU) 10/2011.

In addition, it will need to follow the EN 13432 regarding the conditions to fulfil to be considered compostable.

4.8.1.3. Essential attributes for manufacturing

For the manufacturing of the lidding films from the bioplastic resin, a third-party transformer will be hired. The idea is to produce standard size lidding films ready to seal the trays described below. The production is expected to follow a standard process for lids, but it may change depending on the initial algae by-product nature and transformation process. To achieve a good lid product, it is very important for the bioplastic resin to be as homogenous as possible. Regarding the production cost, it is still early to estimate it, as it may vary significantly depending on the starting product composition and characteristics.

Regarding the technical characteristics of the final lid, it is expected to be transparent, resistant and odourless.

4.8.1.4. Quantities to be produced and specific amounts of required ingredients

As the packaging solutions are obtained from algae residual fractions, in this case, the required ingredient amounts have not been calculated, and the production of the bioplastic resin will

be adapted depending on the algae residual fractions obtained. However, to perform the required tests to evaluate the product quality and behaviour, a minimum number of packaging units will be necessary. It has been estimated that for the production and characterization test, a minimum of 10 units of packaging will be necessary, which are expected by November of 2025. Later, 50 units have been calculated to be necessary for packaging food tests and sensory tests (November 2026). For the industrial stage, the production of 100 units is expected (November 2026), while for testing the consumer acceptance, another 100 units will be necessary (2027). Overall, a minimum production of 1000 meters of lid will be necessary.

4.8.1.5. Methods and criteria for assessing performance

To assess the performance of the product, several tests will be necessary:

- Accelerated shelf-life test with the seafood products: it will be necessary to establish the shelf-life of the seafood products packed inside the biobased solutions. The amount of time that the product can be stored inside the INNOAQUA packaging without losing its properties and the adequacy to be consumed will be established. To do so, accelerated shelf-life test will be performed: It is expected for the product to have a shelf-life in a frozen form of at least **18 months inside the packaging**.
- Consumer acceptance: As part of the assessment of the product quality, a sensory (colour, texture, ...) consumer acceptance evaluation will be performed. This evaluation will be performed at the end of the industrial developing stage, with a panel of at least 40 consumers.

The following parameters have been identified as especially relevant for the lidding films: **Odourless, transparency and colour. They need to be in accordance with the technical specifications of the standard plastic lidding films.**

- General characterization, including the assessing of the mechanical properties, the shelf-life of the bioplastic and several migration tests (for food and hazardous substances).

4.8.2. Trays

4.8.2.1. *General description*

The other packaging solution will be tray made from conventional plastic and residual algae fraction which will be made to contain the food products. The trays will be sealed with the lids described above.

The final characteristics and production process of the trays will be adapted depending on the composition and properties of the residual algae fraction, but the residual algae fraction will suppose 25 to 50% of the composition. In addition, other conventional plastic resins such as PE, PP, HDPE or LDPE may be included. As the current product is made of PP, the development bio-based trays will focus on recyclable PP-based products. In addition, we would look for a compostable product.

The format will be a standard tray of 250x180x70mm, which will be sealable.

The development of the trays will be part of T4.4 and will be led by ERANOVA. ERANOVA is a bioplastic compounder and not a bioplastic object producer, meaning that they are producing the bioresins, but are not able to shape them into the final plastic products. Thus, a plastic transformer external from the INNOAQUA project will be needed to shape the final trays.

The trays, together with the lids will be used to assemble a packaging solution which will be tested with some of the INNOAQUA seafood products. The selected products for this test are the vegan fish filets and vegan surimi.

4.8.2.2. *Regulatory aspects and sustainability*

For the sustainability requirements definition, products such as flexible plastic containers, with a carbon footprint of 0.20-0.31 kg CO₂ eq. (depending on the container type) (44) or plastic cups, with a carbon footprint of 0.04 kg CO₂ eq. (45) can serve as references.

For the packaging solutions, several regulations will apply, as these containers will be in contact with food products. The most relevant ones are:

- Good manufacturing practice for materials and articles intended to come into contact with food. Regulation (EC) 1935/2004;

- Plastic materials and articles intended to come into contact with food. Commission Regulation (EU) 10/2011.

In addition, it will need to follow the EN 13432 regarding the conditions to fulfil to be considered compostable.

4.8.2.3. Essential attributes for manufacturing

For the manufacturing of the trays from the bioplastic resin, a third-party transformer will be hired. The idea is to produce standard size trays ready to be sealed with the lidding films described above. The production is expected to follow a standard industrial production for trays, but it may change depending on the initial algae by-product nature and transformation process. To achieve a good tray product, it is very important for the bioplastic resin to be as homogenous as possible. Regarding the production cost, it is still early to estimate it, as it may vary significantly depending on the starting product composition and characteristics.

Regarding the technical characteristics of the final lid, it is expected to be odourless, resistant and similar in aspect as conventional plastic trays.

4.8.2.4. Quantities to be produced and specific amounts of required ingredients

As the packaging solutions are obtained from algae residual fractions, in this case, the required ingredient amounts have not been calculated, and the production of the bioplastic resin will be adapted depending on the algae residual fractions obtained. However, to perform the required tests to evaluate the product quality and behaviour, a minimum number of packaging units will be necessary. It has been estimated that for the production and characterization test, a minimum of 10 units of packaging will be necessary, which are expected by November of 2025. Later, 50 units have been calculated to be necessary for packaging food tests and sensory tests (November 2026). For the industrial stage, the production of 100 units is expected (November 2026), while for testing the consumer acceptance, another 100 units will be necessary (2027). Overall, a minimum production of 300 trays will be necessary.

4.8.2.5. Methods and criteria for assessing performance

In order to assess the performance of the product, several tests will be necessary:

- Accelerated shelf-life test with the seafood products: it will be necessary to establish the shelf-life of the seafood products packed inside the biobased solutions. The amount of time that the product can be stored inside the INNOAQUA packaging without losing its properties and the adequacy to be consumed will be established. To do so, accelerated shelf-life test will be performed: It is expected for the product to have a shelf-life of at least **18 months inside the packaging**.
- Consumer acceptance: As part of the assessment of the product quality, a sensory (colour, texture, smell...) consumer acceptance evaluation will be performed. This evaluation will be performed at the end of the industrial developing stage, with a panel of at least 40 consumers.
The following parameters have been identified as especially relevant for the trays:
Odourless and with a visual aspect close to the original plastic packaging.
- General characterization, including the assessing of the mechanical properties, the shelf-life of the bioplastic and several migration tests (for food and hazardous substances). The injection properties will also be tested.

4.9. Summary table

After the individual description of each of the products that will be developed as part of the INNOAQUA project, Table 4 summarizes, for each product, the total amount to be produced as well as the INNOAQUA ingredients that will incorporate. It is important to note that, for those cases where a range of values was given in the description, the highest value has always been considered to ensure that enough ingredients are being accounted.

Table 4 Summary of the ingredients to be included in each of the INNOAQUA product.

Product category	Product	Total amount (kg)	Microalgae					Macroalgae			Fish by-products
			Protein (%; kg)	Vitamin (%; kg)	PUFA's (%; kg)	Peptides and AA's (%; kg)	Residual fraction (%; kg)	Protein (%; kg)	Agar (%; kg)	Residual fraction (%; kg)	FPH's (%; kg)
Vegan meat analogues	Sausages	9	30%; 2.70		1%; 0.09			30%; 2.70			
	Nuggets	7.6	30%; 2.28		1%; 0.08	5%; 0.38		30%; 2.30			
Vegan fish analogues	Surimi	207	10%; 20.70		2%; 4.14			10%; 20.70	1%; 2.07		
	Fillets	207	10%; 20.70	0.1%; 0.20	2%; 4.14			10%; 20.70	1%; 2.07		
	Burgers	207	10%; 20.70	0.1%; 0.20	1%; 2.07			10%; 20.70	1%; 2.07		
	Battered /breaded fish portions	207	5%; 10.35		1%; 2.07	1%; 2.07	5%; 10.35	5%; 10.35	1%; 2.07		
Powdered meals	Shakes	16.3	80%; 13.04	0.1%; 0.02		5%; 0.82					
	Breakfast bowls	51.5	30%; 15.45	0.1%; 0.05		5%; 2.58					
	Soups	16.3	60%; 9.78	0.1%; 0.02		5%; 0.82					
Snacks	Cookies	31.5	30%; 9.45	0.1%; 0.03	1%; 0.32						
	Gummy bears	0.85		5%; 0.04	0.5%; 0.004				13%; 0.11		
	Energy bars	31.5	30%; 9.45	0.1%; 0.03		5%; 1.58					
	Energy balls	21.5	20%; 4.30	0.1%; 0.02		5%; 1.08					

Product category	Product	Total amount (kg)	Microalgae					Macroalgae			Fish by-products
			Protein (%; kg)	Vitamin (%; kg)	PUFA's (%; kg)	Peptides and AA's (%; kg)	Residual fraction (%; kg)	Protein (%; kg)	Agar (%; kg)	Residual fraction (%; kg)	FPH's (%; kg)
Other vegan products	Shots	5		1%; 0.05	1%; 0.05						
	Bread Spreads	7	30%; 2.10		1%; 0.07			30%; 2.10			
Hybrid products	Fillets	207	5%; 10.35					10%; 20.70	1%; 2.07	2%; 4.14	1%; 2.07
	Nuggets	7.6	30%; 2.28		1%; 0.08			30%; 2.28			5%; 0.38
	Sausages	9	30%; 2.70		1%; 0.09			30%; 2.70			5%; 0.45
	Bread Spreads	7	30%; 2.10		1%; 0.07			30%; 2.10			5%; 0.35
Packaging	Lidding films	1000 meters					25-50%			25-50%	
	Trays	300 units					25-50%			25-50%	
Total amount			158.43	0.66	13.27	9.33	-	107.33	10.46	-	3.25

All these product prototypes will be developed in the kitchen-development stage, and later, at least 10 vegan products will be selected and scaled-up to industrial scale, as well as at least two hybrid products and both packaging products.

5. Description of the ingredients

5.1. General description

In this section, a detailed description for each of the INNOAQUA ingredient used in the products described in section 4 is provided, including the KPIs they must fulfil. The ingredients are divided into subsections, according to the type of biomass they will come from. A short description of the biomass of origin and its expected composition is also presented.

For each ingredient, the description will be divided in the following subsections:

- General description: a definition of the ingredient will be provided, including aspects such as the main attributes, the composition, and its nutritional information.
- Regulatory aspects and sustainability: this subsection describes the European and different local regulations that apply to the product, including details about possible necessary consultations or approval processes. Regarding sustainability, the different aspects that need to be considered for the ingredients extraction technological development and production is listed, in accordance with the final conclusions of T5.1, led by SUSTAINN. Impact estimations of similar ingredients have been looked for within environmental impacts databases (such as Ecoinvent) to know if there is information about them or otherwise, we will have to generate some raw data within the project. This is a task to be done within WP5.

Some of this information is common for all the ingredients. To avoid reiteration, the general description that applies to all ingredients is included in section 5.1.1, while the specific information for each product is included in the subsequent sections.

- Techno functionality requirements: in this section the intended extraction and modification procedure for each ingredient is shortly described. It also includes information regarding the required final format of the ingredients.
- Quantities to be produced and specific amount of required biomass: in this section, starting from the value of the required amount of the ingredient (calculated in Table 4), and considering the expected extraction yield and biomass composition, it is calculated the amount of biomass that will be required to produce the ingredient.

- Methods and criteria for assessing performance: it lists and describe all the tests that will be necessary to perform to the ingredients to make sure that they fulfil the KPIs and the quality criteria. It may include the test to determine the shelf-life, biological test to assess safety measurements and several characterization test to verify the nutritional properties.

5.1.1. Regulation and sustainability aspects

Regarding regulation and sustainability aspects, the same criteria as for the seafood products and packaging solutions apply. Thus, both the general regulations and sustainability requirements already stated for the products apply and can be checked in section 4.1.1.

5.2. Ingredients obtained from microalgae

Five of the INNOAQUA ingredients will be obtained from microalgae biomass. These ingredients are proteins, PUFA's and carotenoids rich oil, hydrophilic vitamins, peptides and amino acids and the residual fraction.

Three different microalgae biomass will be produced and processed, each one containing a different microalgae specie: *Chlorella sorokiniana*, *Phaeodactylum tricornutum* and *Nannochloropsis oculata*. The reason to choose three different microalgae of origin is the different composition of each of them. *Chlorella sorokiniana* is well known for its high protein and lutein content, as well as a good B-vitamin profile and content. *Phaeodactylum tricornutum* is known to produce large amounts of carotenoids and PUFA's, among them, the most relevant are the omega-3 EPA and DHA. *Nannochloropsis oculata* is especially relevant for the production of PUFA's, as it can produce large amounts of EPA, but also for the production of carotenoids. While processing the three biomasses separately and mixing the obtained extracts together, we expect to be able to obtain high quality ingredients, containing an excellent and complementary nutritional profile.

As part of T3.1 (WP3), a first initial batch of the biomasses is being characterized, and so, a first estimation of the expected compositions has been provided. The values are listed in the Table 5, as it will be useful to estimate the required amounts of biomass to be produced along the project. These values must be taken as a first estimation and not a final value and are going to be used only as calculating references. All the values are expressed in percentage in dried

weight. The vitamin content is not yet available, but it is expected to be in the order of a few mg/g of biomass (46; 47).

Table 5 Composition of the microalgae biomass

Biomass	Moisture (%)	Protein (% DW)	Carbohydrates (% DW)	Total FUPA's (% DW)	Total carotenoids (% DW)
<i>C. sorokiniana</i>	79	43	33	8	1
<i>P. tricornutum</i>	77	31	15	6	0.6
<i>N. oculata</i>	78	36	14	6	0.3

Regarding the biomass production, it is important to note that it will also follow a sustainable approach, looking for the minimization of water and energy consumption and waste generation. The study and minimization of the sustainable impact will be performed as part of WP5.

In terms of regulation, several laws need to be considered during the microalgae biomass production. They include:

- The waste framework directive. 2008/98/EC,
- The water framework directive: 2000/60/EC,
- The Water Reuse Regulation for irrigation. Regulation (EU) 2020/741,
- The animal by-products regulation. Regulation (EC) 1069/2009,
- The animal health law. Regulation (EU) 2016/429,
- The animal welfare directive. 98/58/EC,
- The Nagoya Protocol. Regulation (EU) 511/2014.

It is important to note that as part of the INNOAQUA project, the cultivation of microalgae integrated with a RAS-IMTA systems with salmonids will be performed, thus several regulations regarding animal welfare are also considered.

5.2.1. PUFA's and carotenoids rich oil

5.2.1.1. General description

The first ingredient is an oil rich in carotenoids and PUFA's. It will be obtained from the lipidic-rich fraction of the three microalgae, to contain a mixture of the PUFA's and carotenoids produced by the three species. As the carotenoids have a strong coloration, the product is expected to have a green or brownish intense colour. The flavour can also be strong, and we will intend to neutralize it as much as possible.

The goal would be to have an ingredient with a good omega-3 and omega-6 profile, containing a high concentration of EPA and DHA, as well as alpha-linoleic acid (the last provided mostly by *Chlorella sorokiniana*). A high concentration of bioactive carotenoids will be also expected, including lutein (from *Chlorella sorokiniana*), and fucoxanthin (from *Phaeodactylum tricornutum*).

5.2.1.2. Regulatory aspects and sustainability

There are not much sustainability EPD references for this kind of ingredients. However, it may be useful to compare it with products such as olive oil (48), with carbon footprints between 1,91-3.64 kg CO₂ eq. (depending on the product and the packaging format) or omega-3 rich products for aqua animal feed (49), with a carbon footprint of 2.50 kg CO₂ eq.

Regarding the regulatory aspects, those listed in section 4.1.1 apply to this product.

5.2.1.3. Techno functionality requirements

The PUFA's and carotenoid rich oil will be obtained from the separation of the lipidic fraction after the microalgae cell disruption. A purification step after the separation may be necessary. Due to the lipidic characteristics of this ingredient, the incorporation of preservatives and antioxidants may be necessary, as it will be important for the ingredient to have a shelf-life as long as possible.

5.2.1.4. *Quantities to be produced and specific amount of required biomass*

According to the description of the different seafood products (see above), **the total required amount of this ingredient is 13,3 kg**. For the first stage, the kitchen development, an initial batch of 0.6 kg of PUFA's and carotenoids rich oil will be necessary around M20 (January 2025). To produce enough seafood products for their shelf-life determination, another 0.2 kg of the ingredient will be needed, and later, for the industrial production of the seafood products, a total of 12.5 Kg of the oil will be necessary.

In addition, an extra batch of the ingredient will be necessary for its own characterization and shelf-life determination, namely 1 kg more. And an extra amount will be also consumed during the development of the extraction and separation procedure and the industrial process optimization. It is also important to note that calculations are being performed assuming that all the seafood products will reach the industrial stage, which is not going to be the case, as only 14 out of the 21 prototypes will reach the end of the industrial stage.

Considering that the expected extraction yield is of a minimum of 60%, and tacking into account the microalgae biomass composition (see Table 5), the planned production of 40 kg of each strain plus the final industrial production of 300 Kg (DW) seems just enough to fulfil the expected needs.

5.2.1.5. *Methods and criteria for assessing performance*

To assess the performance of the ingredients, several tests will be necessary:

- Accelerated shelf-life test: it will be necessary to establish the amount of time that the ingredient can be stored without losing its properties and the adequacy to be consumed. To do so, accelerated shelf-life test will be performed by the ingredient producer. It is expected for the oil to have a shelf-life of at least **6 months**.
- General characterization, including the assessing of iodine and heavy metals content, to make sure that they are according to the regulations, as well as biological safety tests. The composition of the ingredient will also be determined. Thus, the determination of the total moisture, PUFA's content and profile, carotenoids content and profile and total protein content will be determined.

5.2.2. Proteins

5.2.2.1. *General description*

The second ingredient is the protein extract. It will be obtained from the separation of the aqueous phase after the microalgae biomass disruption, and the subsequent separation of the vitamins and small molecules from the proteins. The extract may be purified or even fractionated in different parts regarding the protein sizes, to obtain different protein concentrates with different techno functional properties.

The preferred format would be a dried powder, but for the formulation of certain seafood products, an aqueous format may also be considered. In any case, the ingredient needs to have a high solubilization degree, as well as a flavour as neutral as possible. Most of the seafood producers also highlighted that the absence of strong green or brownish colours is fundamental, so a protein as colourless as possible will be looked for. To achieve this, an excellent separation of the protein from the chlorophyll and other colourful molecules will be necessary.

Regarding its nutritional attributes, it is important for the ingredient to have a very high protein content. The amino acid profile of the ingredient will also be important, and a good profile and content of essential amino acids is expected. The proteins are also expected to be of high digestibility.

5.2.2.2. *Regulatory aspects and sustainability*

There are not much sustainability EPD references for alternative protein ingredients. However, a Product Category rule for functional food ingredients is being developed, and it will include some data regarding protein isolates (50).

Regarding the regulatory aspects, those listed in section 4.1.1 apply to this product.

5.2.2.3. *Techno functionality requirements*

The protein ingredient will be obtained from the processing of the aqueous fraction obtained after the biomass disruption. This aqueous fraction will be further purified to separate and fraction the proteins. It will be preferably done by ultrafiltration with membranes of different

pore sizes. For the maximum elimination of pigments other steps may be needed, which may include some solvent washes or hydrolysis steps.

As the preferred format for the protein concentrates is in dried form, some drying systems will need to be included in the process. In addition, and especially to produce vegan meat and fish analogues, the protein may be additionally texturized, with wet or dry extrusion processes or other possible methods. The fractionation of the protein in different size-selected parts may also contribute in the obtention of ingredients with the desired techno functional properties.

5.2.2.4. Quantities to be produced and specific amount of required biomass

According to the description of the different seafood products (see above), **the total required amount of this ingredient is 158.4 kg**. For the first stage, the kitchen development, an initial batch of 11.7 kg of microalgae proteins will be necessary around M20 (January 2025). To produce enough seafood products for their shelf-life determination, another 2.6 kg of the ingredient will be needed, and later, for the industrial production of the seafood products, a total of 144.2 kg of microalgae proteins will be necessary.

In addition, an extra batch of the ingredient will be necessary for its own characterization and shelf-life determination, namely 1 kg more. And an extra amount will be also consumed during the development of the extraction and separation procedure and the industrial process optimization. It is also important to note that calculations are being performed assuming that all the seafood products will reach the industrial stage, which is not going to be the case, as only 14 out of the 21 prototypes will reach the end of the industrial stage. Thus, we can assume that around 120 kg of microalgae protein will be necessary.

Considering that the expected extraction yield is of a minimum of 65%, assuming 120 kg of required microalgae protein and an average of a microalgae protein content of 40% (see Table 5), the required amount of biomass would be of 460 kg. The planned production of 40kg of each strain of microalgae plus the final industrial production of 300 kg (DW) seems just below the expected needs. Thus, we will need to consider several strategies to re-adjust:

- Option A: slightly increase the microalgae production to meet the protein needs (40 kg more).

- Option B: reduce the production amounts of several seafood products to adjust to the available microalgae protein.
- Option C: maintain the planned microalgae and seafood products production but reduce the formulated amount of microalgae protein into the seafood products by mixing it with other vegan protein sources (soy, pea, chickpea, etc).

5.2.2.5. *Methods and criteria for assessing performance*

To assess the performance of the ingredients, several tests will be necessary:

- Accelerated shelf-life test: it will be necessary to establish the amount of time that the ingredient can be stored without losing its properties and the adequacy to be consumed. To do so, accelerated shelf-life test will be performed by the ingredient producer. It is expected for the microalgae proteins to have a shelf-life of at least **12 months**.
- General characterization, including the assessing of iodine and heavy metals content, to make sure that they are according to the regulations, as well as biological safety tests. The composition of the ingredient will also be determined. Thus, the determination of the total moisture, total protein content, protein sizes, amino acids profile, PUFA's content and carotenoids content will be determined.

It may also be necessary to characterize the techno functional properties of one or more protein fractions, in terms of emulsifying and foaming properties, solubility, or gelling ability.

5.2.3. Hydrophilic vitamins

5.2.3.1. *General description*

The third ingredient obtained from microalgae is the hydrophilic vitamins extract. It will be obtained from the separation of the aqueous phase after the microalgae biomass disruption, and the subsequent separation of the vitamins and small molecules from the proteins. Consequently, it will be an aqueous fraction rich in vitamins, amino acids, peptides and other small molecules.

The preferred format would be a dried powder, but for the formulation of certain seafood products, an aqueous format may also be considered. In any case, the ingredient needs to have a high solubilization degree, as well as a flavour as neutral as possible.

Regarding its nutritional attributes, it is important for the ingredient to have a good vitamin profile. The ingredient will be a mixture of the extracts of the three biomasses, so the vitamins produced by the three microalgae will be incorporated. The presence of vitamins of the B-group, and especially B12, is relevant because the common deficit in people following a vegan diet. *Chlorella's* are known for having high levels of these vitamins, and thus, a high content of vitamin B12 and other B-group vitamins is expected in the microalgae hydrophilic vitamins extract.

5.2.3.2. Regulatory aspects and sustainability

Regarding sustainability references, we were not able to find any comparable reference.

Regarding the regulatory aspects, those listed in section 4.1.1 apply to this product. As it is a vitamins extract, it is important to consider the Regulation (EC) 1925/2006, about the addition of vitamins and minerals to food products. This regulation includes an annex that lists all the vitamins and minerals allowed to be included in food products.

5.2.3.3. Techno functionality requirements

The hydrophilic vitamin ingredient will be obtained from the processing of the aqueous fraction obtained after the biomass disruption. This aqueous fraction will be further purified to separate the vitamins and other small molecules from the proteins. It will be preferably done by ultrafiltration with membranes of different pore sizes.

As the preferred format for the vitamins ingredient is in dried form, some drying systems will need to be included in the process. The shelf-life of the product will also need to be tested, and if it is not long enough, the addition of preservatives and antioxidants may be considered.

5.2.3.4. Quantities to be produced and specific amount of required biomass

According to the description of the different seafood products (see above), **the total required amount of this ingredient is 660 g**. For the first stage, the kitchen development, an initial batch of 50 g of microalgae vitamins will be necessary around M20 (January 2025). To produce

enough seafood products for their shelf-life determination, another 20 g of the ingredient will be needed, and later, for the industrial production of the seafood products, a total of 610 g of microalgae proteins will be necessary.

In addition, an extra batch of the ingredient will be necessary for its own characterization and shelf-life determination, namely 500 g more. And an extra amount will be also consumed during the development of the extraction and separation procedure and the industrial process optimization. It is also important to note that calculations are being performed assuming that all the seafood products will reach the industrial stage, which is not going to be the case, as only 14 out of the 21 prototypes will reach the end of the industrial stage.

The vitamin content of the original biomasses has yet to be determined, and thus, the value that we will estimate may happen to be far from the reality. However, to calculate the amount of biomass necessary, we can assume a mean vitamin content of 2 mg/ g of biomass (47; 46). Considering that the expected extraction yield is of a minimum of 60%, the calculated biomass requirement exceeds the planned microalgae biomass production by 2-3-fold. Thus, we will need to consider several strategies to re-adjust:

- Option A: slightly increase the microalgae production to meet the vitamins needs (40 kg more).
- Option B: reduce the production amounts of several seafood products to adjust to the available microalgae vitamins.
- Option C: maintain the planned microalgae and seafood products production but reduce the formulated amount of microalgae vitamin into the seafood products.

For the case of the vitamins, the option C seems the most viable.

5.2.3.5. *Methods and criteria for assessing performance*

To assess the performance of the ingredients, several tests will be necessary:

- Accelerated shelf-life test: it will be necessary to establish the amount of time that the ingredient can be stored without losing its properties and the adequacy to be consumed. To do so, accelerated shelf-life test will be performed by the ingredient producer. It is expected for the microalgae vitamins to have a shelf-life of at least **6 months**.

- General characterization, including the assessing of iodine and heavy metals content, to make sure that they are according to the regulations, as well as biological safety tests. The composition of the ingredient will also be determined. Thus, the determination of the total moisture, total vitamin content and profile and total protein content will be determined.

5.2.4. Peptides and amino acids

5.2.4.1. *General description*

The next ingredient is a peptides and amino acids fraction. It will be an aqueous ingredient, rich in amino acids and peptides that will be produced from the solid fraction obtained after the biomass disruption. This solid fraction will be treated with an enzymatic hydrolysis, which will turn the non-soluble proteins into water-soluble small peptides and free amino acids. In this way, we would be able to benefit from the non-soluble proteins that we were not able to extract directly in the aqueous phase after the cell disruption. After the enzymatic hydrolysis, it may be necessary to further purify the sample, for example, using ultrafiltration techniques. The obtained ingredient is expected to have a very good amino acid profile, and to be colourless and with a good flavour.

5.2.4.2. *Regulatory aspects and sustainability*

Regarding sustainability references, we were not able to find any comparable reference.

Regarding the regulatory aspects, those listed in section 4.1.15.1.1 apply to this product.

5.2.4.3. *Techno functionality requirements*

The ingredient will be obtained from the processing of the solid fraction obtained by ALGEMY after the biomass disruption. This aqueous fraction will be further processed by LEITAT to recover the non-soluble proteins left in the solid pellet. LEITAT will apply a specially designed enzymatic hydrolytic (SDEH) treatment to cut the protein into short-length peptides and free amino acids, which will be then soluble in water, and will be separated from the remaining solid fractions (rich in fibre and other polysaccharides).

The ingredient will be obtained as a liquid, but a drying process to obtain a solid powder may be considered. In any way, the ingredient must have an easy solubility for its incorporation in the food formulations.

5.2.4.4. Quantities to be produced and specific amount of required biomass

According to the description of the different seafood products (see above), **the total required amount of this ingredient is 9.3 kg**. For the first stage, the kitchen development, an initial batch of 0.4 kg of microalgae peptides and amino acids will be necessary around M20 (January 2025). To produce enough seafood products for their shelf-life determination, another 0.1 kg of the ingredient will be needed, and later, for the industrial production of the seafood products, a total of 8.8 kg of microalgae proteins will be necessary.

In addition, an extra batch of the ingredient will be necessary for its own characterization and shelf-life determination, namely 1 kg more. And an extra amount will be also consumed during the development of the extraction and separation procedure and the industrial process optimization. It is also important to note that calculations are being performed assuming that all the seafood products will reach the industrial stage, which is not going to be the case, as only 14 out of the 21 prototypes will reach the end of the industrial stage.

Considering that the expected extraction yield is of around 20%, that the protein fraction left in the solid pellet is expected to be around 30% of the total protein, and tacking into account the microalgae biomass composition (see Table 5), the planned production of 40 kg of each strain plus the final industrial production of 300 kg (DW) seems just enough for the expected needs.

5.2.4.5. Methods and criteria for assessing performance.

To assess the performance of the ingredients, several tests will be necessary:

- Accelerated shelf-life test: it will be necessary to establish the amount of time that the ingredient can be stored without losing its properties and the adequacy to be consumed. To do so, accelerated shelf-life test will be performed by the ingredient producer. It is expected for the microalgae peptides and amino acids to have a shelf-life of at least **6 months**.

- General characterization, including the assessing of iodine and heavy metals content, to make sure that they are according to the regulations, as well as biological safety tests. The composition of the ingredient will also be determined. Thus, the determination of the total moisture, total vitamin content, total protein content and amino acid profile will be determined.

5.2.5. Residual polysaccharides

5.2.5.1. *General description*

The last ingredient obtained from microalgae will be the solid fraction rich in polysaccharides left as the residual fraction after the recovery of all the other ingredients. After the disruption of the microalgae biomass and 3-phase separation, the solid fraction will be enzymatically hydrolysed to recover the peptides and amino acids, as described in the previous section. After this process a solid fraction will remain, which will be rich in polysaccharides, and which will be of no use for the food industry. This residual fraction will constitute the last of the microalgae ingredients, and will be collected by ERANOVA, which together with the macroalgae residual fraction, will apply a treatment to transform this ingredient into a plastic resin, which later will be used to produce the packaging solutions.

This ingredient is expected to be mostly consistent of polysaccharides, with a considerable fibre content. Due to this fibre rich content, the incorporation of small amounts of this ingredient into the formulation of one seafood products (battered/breaded vegan fish portions) is also being considered.

5.2.5.2. *Regulatory aspects and sustainability*

As this ingredient is a by-product obtained after the extraction of all the other ingredients and will later be used as starting material for the production of the plastic resins, the individual sustainability assessment of this ingredient will not be performed and it will be included as part of the plastic resin assessment.

As this ingredient is expected to mostly be used for the development of the packaging solutions, the regulations that will need to be considered will be those related with the packaging, namely regulation (EC) 1935/2004 and Commission Regulation (EU) 10/2011.

However, if it is finally also used in any of the seafood products, all the regulations listed in section 4.1.1 and related with food products will also need to be considered.

5.2.5.3. Techno functionality requirements

As this ingredient is the residual fraction, the techno functionality requirements are limited. The posterior process of obtention of the plastic resin from this product will be highly adapted depending on the composition and characteristics of this residual fraction.

A process of drying the residual polysaccharides fraction may be necessary.

5.2.5.4. Quantities to be produced and specific amount of required biomass

This ingredient is the residual fraction obtained after the extraction of all the other microalgae ingredients. The goal is to be able to valorise this residue and produce some bioplastic-based packaging solutions with it. All the residual fraction obtained is intended to be used, but in any case, no extra biomass will be produced to obtain more residual fraction. Thus, this ingredient is not a limiting factor for the biomass production.

5.2.5.5. Methods and criteria for assessing performance

To assess the performance of the ingredients, several tests will be necessary:

- Accelerated shelf-life test: it will be necessary to establish the amount of time that the ingredient can be stored without losing its properties. To do so, accelerated shelf-life test will be performed by the ingredient producer. It is expected for the microalgae residual fraction to have a shelf-life of at least **12 months**.
- General characterization. The residual fraction will be characterized, as the process to obtain the bioplastic resin strongly depends on its composition and characteristics. The total carbohydrates content will be determined, as well as the amount of remaining proteins. In addition, some other simple test to determine the textural properties may be also required.

5.3. Ingredients obtained from macroalgae

Three of the INNOAQUA ingredients will be obtained from macroalgae biomass. These ingredients are proteins, agar and the residual fraction.

Three different macroalgae biomass will be produced and processed, each one containing a different macroalgae genus: *Gracilaria*, *Porphyra* and *Ulva*. The reason to choose three different macroalgae genus is the different composition of each of them. *Gracilaria* is known for its agar production, as well as a high protein content. *Porphyra* and *Ulva* have a high protein content.

As the macroalgae biomass to be used in the INNOAQUA project has not yet been characterized, some composition values extracted from literature and our previous knowledge are being used for the necessary biomass production calculations (51; 52; 53). These values must be taken as a first estimation and not a final value and are going to be used only as calculating references. All the values are expressed in percentage in dried weight.

Table 6 Bibliographic data of macroalgae biomass composition.

Biomass	Protein (% DW)	Carbohydrates (% DW)	Agar (% DW)	Minerals (% DW)	Lipids (% DW)
<i>Gracilaria</i>	20-45	25-60	11-30	~25	~1.5
<i>Porphyra</i>	25-47	25-44	-	12-28	~2.8
<i>Ulva</i>	10-32	30-56	-	12-37	0.6 - 3.9

Regarding the biomass production, it is important to note that it will also follow a sustainable approach, looking for the minimization of water and energy consumption and waste generation. The study and minimization of the sustainable impact will be performed as part of WP5.

In terms of regulation, several laws need to be considered during the macroalgae biomass production. They include:



- The waste framework directive. 2008/98/EC,
- The water framework directive: 2000/60/EC,
- The Water Reuse Regulation for irrigation. Regulation (EU) 2020/741,
- The animal by-products regulation. Regulation (EC) 1069/2009,
- The animal health law. Regulation (EU) 2016/429,
- The animal welfare directive. 98/58/EC,
- The Nagoya Protocol. Regulation (EU) 511/2014.

It is important to note that as part of the INNOAQUA project, the cultivation of macroalgae integrated with RAS-IMTA systems with sole fish will be performed, thus several regulations regarding animal welfare are also considered.

5.3.1. Proteins

5.3.1.1. *General description*

The first ingredient obtained from macroalgae is the protein extract. It will be obtained with an aqueous extraction of the biomass after cell disruption, and the subsequent separation and purification by means of an ultrafiltration process. The extract may be fractionated in different parts regarding the protein sizes, to obtain different protein isolates or concentrates with different techno functional properties.

The preferred format would be a dried powder, which needs to have a high solubilization degree, as well as a flavour as neutral as possible. Most of the seafood producers also highlighted that the absence of strong green or brownish colours is fundamental, so a protein as colourless as possible will be looked for. To achieve so, an excellent separation of the protein from the chlorophyll and other colourful molecules will be necessary.

Regarding its nutritional attributes, it is important for the ingredient to have a very high protein content, which can vary from 30 to 70% depending on the biomass of origin and the extraction protocol. The amino acid profile of the ingredient will also be important, and a good profile and content of essential amino acids is expected. The proteins are also expected to be of high digestibility.

5.3.1.2. *Regulatory aspects and sustainability*

There are not much sustainability EPD references for alternative protein ingredients. However, a Product Category rule for functional food ingredients is being developed, and it will include some data regarding protein isolates (50).

Regarding the regulatory aspects, those listed in section 4.1.1 apply to this product.

5.3.1.3. *Techno functionality requirements*

The protein ingredient will be obtained from the processing of the aqueous fraction obtained after the biomass disruption. This aqueous fraction will be further purified to separate and fraction the proteins. It will be preferably done by ultrafiltration with membranes of different pore sizes.

As the preferred format for the protein concentrates is in dried powdered form, some drying systems will need to be included in the process. In addition, and especially to produce vegan meat and fish analogues, the protein may be additionally texturized, with wet or dry extrusion processes or other possible methods. The fractionation of the protein in different size-selected parts may also contribute in the obtention of ingredients with the desired techno functional properties.

5.3.1.4. *Quantities to be produced and specific amount of required biomass*

According to the description of the different seafood products (see above), the **total required amount of this ingredient is 107.3 kg**. For the first stage, the kitchen development, an initial batch of 9.5 kg of macroalgae proteins will be necessary around M20 (January 2025). To produce enough seafood products for their shelf-life determination, another 1.7 kg of the ingredient will be needed, and later, for the industrial production of the seafood products, a total of 96.2 kg of macroalgae proteins will be necessary.

In addition, an extra batch of the ingredient will be necessary for its own characterization and shelf-life determination, namely 1 kg more. An extra amount will be also consumed during the development of the extraction and separation procedure and the industrial process optimization. It is also important to note that calculations are being performed assuming that all the seafood products will reach the industrial stage, which is not going to be the case, as only 14 out of the 21 prototypes will reach the end of the industrial stage.

Considering a total requirement of 80 kg and an expected extraction yield of a minimum of 65%, and taking into account the macroalgae biomass composition (see Table 6; we take a cautious value from the lowest side of the range, namely 20%), **the total production of macroalgae biomass (including the three genus) should be of at least 615 kg (DW)**. Part of this amount is already being produce as the first small batch (50 kg), and the other 515 kg will need to produce during the industrial stage. However, the calculations will need to be revised in the following year, when the actual protein content of the biomass will be known, and a more accurate formulation of the seafood products would have been performed.

5.3.1.5. *Methods and criteria for assessing performance*

To assess the performance of the ingredients, several tests will be necessary:

- Accelerated shelf-life test: it will be necessary to establish the amount of time that the ingredient can be stored without losing its properties and the adequacy to be consumed. To do so, accelerated shelf-life test will be performed by the ingredient producer. It is expected for the macroalgae proteins to have a shelf-life of at least **12 months**, but it may vary depending on the storage conditions.
- General characterization, including the assessing of iodine and heavy metals content, to make sure that they are according to the regulations, as well as biological safety tests. The composition of the ingredient will also be determined. Thus, the determination of the total moisture, total protein content and ash content will be determined.

5.3.2. Agar

5.3.2.1. *General description.*

Another ingredient obtained from macroalgae is the agar. Agar is a gelatinous substance made of a mixture of polysaccharides (agarose and agaropectins) and that is naturally obtained from the cell wall of some red algae. In the INNOAQUA project, the cultivated macroalgae *Gracilaria* will produce agar, which will be extracted and used as an ingredient for the formulation of some of the seafood products. Agar stands out for its jellifying properties, while having a neutral flavour and colour. It will be obtained from processing the solid fraction resulted after

the *Gracilaria* biomass cell disruption. Specifically, the agar will be extracted with alkaline hot solutions, and it will later be precipitated and dried to obtain a powder or flakes.

5.3.2.2. *Regulatory aspects and sustainability*

Regarding sustainability references, we were not able to find any comparable reference.

Regarding the regulatory aspects, those listed in section 4.1.1 apply to this product.

5.3.2.3. *Techno functionality requirements*

The *Gracilaria* biomass will first need to be subjected to cell disruption to release all the cell components. Later, all the components will be separated. First with a solid-liquid separation, which will allow to separate the soluble proteins, vitamins and minerals from the non-soluble compounds. The solid fraction, containing the non-soluble compounds will later be subsequently treated with hot alkaline solutions to solubilize and extract the agar. This agar will then be precipitated from the alkaline solutions to obtain a solid ingredient, which will be dried into a powder or flakes.

The ingredient, once mixed in the formulations will need to have a jelly-like texture (high jellifying properties), a neutral flavour and a colourless appearance.

5.3.2.4. *Quantities to be produced and specific amount of required biomass*

According to the description of the different seafood products (see above), **the total required amount of this ingredient is 10.5 kg**. For the first stage, the kitchen development, an initial batch of 0.3 kg of macroalgae agar will be necessary around M20 (January 2025). To produce enough seafood products for their shelf-life determination, another 0.1 kg of the ingredient will be needed and, later, for the industrial production of the seafood products, a total of 10 kg of macroalgae agar will be necessary.

In addition, an extra batch of the ingredient will be necessary for its own characterization and shelf-life determination, namely 1 kg more. An extra amount will be also consumed during the development of the extraction and separation procedure and the industrial process optimization. It is also important to note that calculations are being performed assuming that all the seafood products will reach the industrial stage, which is not going to be the case, as only 14 out of the 21 prototypes will reach the end of the industrial stage.

Considering a total requirement of Agar of 10 kg and an expected extraction yield of a minimum of 60% , and tacking into account the *Gracilaria* biomass composition (see Table 6; we take a cautious value from the lowest side of the range, namely 11%), **the total production of *Gracilaria* biomass should be of at least 152 kg in order to be able to supply the required Agar. Thus, from the total production of 615 kg of macroalgae biomass required, at least 152 kg (DW) will need to be from *Gracilaria*.** However, the calculations will need to be revised in the following year, when the actual agar content of the biomass will be known, and a more accurate formulation of the seafood products would have been performed.

5.3.2.5. *Methods and criteria for assessing performance*

To assess the performance of the ingredients, several tests will be necessary:

- Accelerated shelf-life test: it will be necessary to establish the amount of time that the ingredient can be stored without losing its properties and the adequacy to be consumed. To do so, accelerated shelf-life test will be performed by the ingredient producer. It is expected for the agar to have a shelf-life of at least **12 months**, but it may vary depending on the storage conditions.
- General characterization, including the assessing of iodine and heavy metals content, to make sure that they are according to the regulations, as well as biological safety tests. The composition of the ingredient will also be determined. Thus, the determination of the total moisture, total carbohydrates, total protein content and ash content will be determined.

5.3.3. Residual mix of polysaccharides and proteins

5.3.3.1. *General description*

The last ingredient obtained from macroalgae will be the solid fraction rich in polysaccharides left as the residual fraction after the recovery of all the other ingredients. After the disruption of the macroalgae biomass and the solid-liquid separation, the solid fraction will be treated to recover the agar, as described in the previous section. After this process a solid fraction will remain, which will be rich in polysaccharides, and which will be of no use for the food industry.

However, if in this step the lipid content of the solid is considerable, a lipid-washing steps with solvents may be included in the process to recover this compound.

This residual fraction will constitute the last of the macroalgae ingredients, and will be collected by ERANOVA, which together with the macroalgae residual fraction, will apply a treatment to transform this ingredient into a plastic resin, which later will be used to produce the packaging solutions.

This ingredient is expected to be mostly consistent of polysaccharides and non-soluble proteins, with a considerable fibre content. Due to this fibre rich content, the incorporation of small amounts of this ingredient into the formulation of one seafood products (hybrid fish filets) is also being considered.

5.3.3.2. Regulatory aspects and sustainability

As this ingredient is a by-product obtained after the extraction of all the other ingredients and will later be used as starting material for the production of the plastic resins, the individual sustainability assessment of this ingredient will not be performed and it will be included as part of the plastic resin assessment.

As this ingredient is expected to mostly be used for the development of the packaging solutions, the regulations that will need to be considered will be those related with the packaging, namely regulation (EC) 1935/2004 and Commission Regulation (EU) 10/2011.

However, if it is finally also used in any of the seafood products, all the regulations listed in section 4.1.1 and related with food products will also need to be considered.

5.3.3.3. Techno functionality requirements

As this ingredient is the residual fraction, the techno functionality requirements are limited. The posterior process of obtention of the plastic resin from this product will be highly adapted depending on the composition and characteristics of this residual fraction.

A process of drying the residual polysaccharides fraction may be necessary.

5.3.3.4. Quantities to be produced and specific amount of required biomass

This ingredient is the residual fraction obtained after the extraction of all the other macroalgae ingredients. The goal is to be able to valorise this residue and produce some bioplastic-based

packaging solutions with it. So, all the residual fraction obtained is intended to be used, but in any case, no extra biomass will be produced to obtain more residual fraction. Thus, this ingredient is not a limiting factor for the biomass production.

5.3.3.5. *Methods and criteria for assessing performance*

To assess the performance of the ingredients, several tests will be necessary:

- Accelerated shelf-life test: it will be necessary to establish the amount of time that the ingredient can be stored without losing its properties. To do so, accelerated shelf-life test will be performed by the ingredient producer. It is expected for the macroalgae residual fraction to have a shelf-life of at least 12 months.
- General characterization. The residual fraction will be characterized, as the process to obtain the bioplastic resin strongly depends on its composition and characteristics. The total carbohydrates content will be determined, as well as the moisture and the amount of remaining proteins. The lipid content will also be measured and, if it is high, some lipid-washing steps with solvents may be included in the process. In addition, some other simple test to determine the textural properties may be also required.

5.4. Ingredients obtained from fish waste

The last source of biomass are the fish by-products. Three different sources of biomass will be exhaustively characterized (T3.1) and subjected to the first processing tests to obtain the fish protein hydrolysates. The most promising source among the three will be later selected and this source is the one that will be used for its processing and up-scaling to industrial level to produce the required amounts of the ingredient. The three different sources are:

- salmon skins: produced as a by-product on the VIKINGAQUA facilities;
- Salmon heads: produced as by-product on the VIKINGAQUA facilities;
- Non-compliant products from surimi manufacturing: produced as by-products on the PESCANOVA facilities.

All the three sources have in common that, despite being considered by-products, still contain a lot of protein, which, with the adequate processing, should be available for its recovery and utilization as food ingredient. In addition to the proteins, the salmon skins and heads are expected to be very rich in lipids, while the non-compliant surimi sticks may contain high levels of starch. Thus, the challenge will be to separate the desired proteins from the rest of the products.

As part of T3.1 (WP3), a first initial batch of the by-side products is being characterized, and so, a first estimation of the expected compositions has been provided. The values will be listed in the Table 7, as it will be useful to estimate the required amounts of biomass to be produced along the project. These values must be taken as a first estimation and not a final value and are going to be used only as calculating references. All the values are expressed in percentage in dried weight.

Table 7 Composition of the fish by-products.

	Moisture (%)	Protein (% DW)	Lipids (% DW)
Salmon Heads	55	23	56
Salmon Skins	53	30	70
Non-compliant surimi sticks	76	28	<5

5.4.1. Functional FPH

5.4.1.1. General description

Three different fish by-products will be investigated in the context of their usability for the production of fish protein hydrolysate. They will be mildly grounded and then subjected to an ultrasound-assisted treatment to facilitate the protein extraction, if required. Later, proteins will be extracted through a mild enzymatic hydrolysis to preserve the protein functional properties and to improve the taste and odour of the protein hydrolysates. The oils will be separated from the mixture, and the obtained protein hydrolysate will be dried to obtain a

powdered ingredient. Among the different fish protein hydrolysates, the most promising one will be selected to be scaled up and formulated in food products.

This ingredient will be of high nutritional value and rich in proteins. The goal is to obtain a product with a good solubility, and a flavour as neutral as possible. The product is also expected to have bio-functional properties such as antioxidant capacity, antihypertensive activity and anti-inflammatory properties.

5.4.1.2. Regulatory aspects and sustainability

Regarding sustainability references, we were not able to find any comparable reference.

Regarding the regulatory aspects, those listed in section 4.1.1 apply to this product.

5.4.1.3. Techno functionality requirements

The fish protein hydrolysates will be extracted from the selected fish by-product by means of a mild enzymatic hydrolysis process assisted with ultrasounds if required and will later be dried in order to obtain a powdered ingredient.

For the ingredient to be easily incorporated into the seafood formulations, it is required for it to have a good solubility and water holding capacity. It is also very important for the ingredient to have a flavour as neutral as possible and a mild colour.

5.4.1.4. Quantities to be produced and specific amount of required biomass

According to the description of the different seafood products (see above), **the total required amount of this ingredient is 3.3 kg**. For the first stage, the kitchen development, an initial batch of 0.7 kg of FPH's will be necessary around M20 (January 2025). To produce enough seafood products for their shelf-life determination, another 0.1 kg of the ingredient will be needed, and later, for the industrial production of the seafood products, a total of 2.5 kg of FPH's will be necessary.

In addition, an extra batch of the ingredient will be necessary for its own characterization and shelf-life determination, namely 300 g more in total (distributed among the kitchen and industrial stages). And an extra amount will be also consumed during the development of the extraction and separation procedure and the industrial process optimization. It is also important to note that calculations are being performed assuming that all the seafood

products will reach the industrial stage, which is not going to be the case, as only 14 out of the 21 prototypes will reach the end of the industrial stage.

Considering a total requirement of 3.5 kg and an expected extraction yield of a minimum of 60%, and tacking into account the fish by-products composition (see Table 7), **the total necessary fish by-products will be of (in wet weight) at least 40 kg of salmon skins, 56 kg of salmon heads, or 89 kg of non-compliant surimi sticks**, depending on which one will be chosen.

5.4.1.5. Methods and criteria for assessing performance

To assess the performance of the ingredients, several tests will be necessary:

- Accelerated shelf-life test: it will be necessary to establish the amount of time that the ingredient can be stored without losing its properties and the adequacy to be consumed. To do so, accelerated shelf-life test will be performed by the ingredient producer, at the end of the industrial stage. It is expected for the FPH's to have a shelf-life of at least **6 months**.
- General characterization, including the assessing of iodine and heavy metals content, to make sure that they are according to the regulations, as well as biological safety tests. The composition of the ingredient will also be determined. Thus, the determination of the total moisture, ash content, lipid content, total protein content and the peptides and amino acid profile will be determined. In addition, several tests will be performed to assess the techno-functional properties (solubility, water holding capacity, oil holding capacity, viscosity and other rheological properties, emulsifying & foaming properties, gelling ability, fat binding capacity, sensory properties...) and the bioactivities (antioxidant capacity, antihypertensive assessment, and anti-inflammatory assessment).

5.5. Other

This last category includes the only ingredient that is not produced directly from a biomass but from other ingredients. It is the plastic resin, obtained after the processing of the microalgae and macroalgae residual fractions.

5.5.1. Plastic resin

5.5.1.1. *General description*

The biobased plastic resin will be obtained after the processing of the microalgae and macroalgae fractions and its mixing with other additives and plasticizers to obtain a resin with the right properties. The obtained plastic resins will be later extruded to create the compound, small round plastic granules. This compound is the product that can then be transformed into the different packaging solutions.

The biobased plastic resin is expected to be resistant enough, also to aging and to have the right flexibility and density for its application.

5.5.1.2. *Regulatory aspects and sustainability*

For the sustainability requirements definition, information on similar products have been looked for to compare. EPD data of different plastic resins could be found (54; 55; 56), with carbon footprints of 1.97, 2.43 and 0.78 kg CO₂ eq., respectively.

As this ingredient will only be used for the development of the packaging solutions, the regulations that will need to be considered will be those related with the packaging, namely regulation (EC) 1935/2004 and Commission Regulation (EU) 10/2011.

5.5.1.3. *Techno functionality requirements*

ERANOVA will select the most suitable raw materials apart from the remaining algae fractions (additives, plasticizers, polyolefins, etc.) and will design an experimental plan to produce the best biobased resin formulations. It will be necessary to consider the composition and characteristics of the microalgae and macroalgae residual fractions, as they can largely affect the resin properties as well as the plasticizing kinetics. The resins will later be screw extruded in order to produce the compounds. To do so, the operating conditions, such as extruder temperature, pressure, flow rate and energy consumption, will be optimized.

5.5.1.4. *Quantities to be produced and specific amount of required biomass*

As this ingredient will be obtained after the processing and valorisation of the algae residual fractions, it is not necessary to calculate the specific amount of biomass required. The goal is to be able to valorise this residue and produce some bioplastic-based packaging solutions with

it. So, all the residual fraction obtained is intended to be used, but in any case, no extra biomass will be produced to obtain more bio-based resin. Thus, this ingredient is not a limiting factor for the biomass production.

5.5.1.5. Methods and criteria for assessing performance

To assess the performance of the ingredients, several tests will be necessary:

- Accelerated shelf-life test: it will be necessary to establish the amount of time that the resin can be stored without losing its properties. To do so, accelerated shelf-life test will be performed by the ingredient producer.
- General characterization, including the assessing of the biological safety requirements, the absence of hazardous substances and some food migration tests. In addition, several test will be performed to determine the resin density, melt flow index, elasticity modulus, resistance at break traction and flexion, glass transition temperature, melting temperature and viscosity. The Izod and Charpy impact tests, the Brinell hardness test, a water absorption test, a thermic resistance test and the Dart impact test will also be performed.

6. Conclusions

For each INNOAQUA seafood product and packaging solution, a general description has been provided, including the desired product appearance and flavour, nutritional attributes and manufacturing techniques and processes that will be necessary. Furthermore, information regarding sustainability, regulatory and consumer acceptance considered to be relevant for the proper development of the product have also been provided. The expected ingredients to be used in the formulation have been listed, with particular emphasis for the INNOAQUA ingredients, for which the required amounts have been estimated.

In section 5, the different INNOAQUA ingredients have been introduced, in a similar way than the products. For each of them, a general description has been provided, including its nutritional attributes and a description of the expected production methodology and techno-functional modifications. The necessary regulatory and sustainability considerations have also been described, as well as the characterization techniques which are expected to be applied to study the ingredients. This section also includes information regarding the biomass of origin, which will be produced as part of WP2. The description of the different biomasses has been provided, with special focus to the nutritional composition. This information, together with the calculated required amounts for each of the ingredients, and the expected extraction yields during the ingredient obtention, has served to estimate how much biomass will be necessary for the smooth project development. For two of the ingredients obtained from microalgae (proteins and vitamins), the calculated required amount of biomass is larger than the expected production. In this case, either the biomass production will need to increase, or the amount of ingredients used decreases (either by reducing the seafood product production or by reducing the formulated microalgae protein amount in the products).

Some of the information included in the document is a preliminary approach, which will later need to be updated. This is the case of the consumer acceptance, regulatory and sustainability information, which will be updated with the outcomes of WP1 and WP5, respectively. Other relevant information that will need to be modified is the production costs, which will be readjusted once more insight on the final product formulation and ingredients production processes is provided by WP3 and WP4. The calculations of the required biomass amounts are



currently also indicative and will also be revised once the actual biomass composition is determined and the seafood product formulations are decided in the kitchen development stage.

Overall, this document, which counts with the collaboration and approval of all the biomass producers from WP2, the ingredient producers from WP3, the seafood and packaging producers from WP4, the consumer acceptance experts from WP1 and the regulatory and sustainability experts from WP5 is expected to serve as guideline for all the involved actors during the development of the different INNOAQUA ingredients and products. The document will be subsequently updated with the new information and knowledge that arises during the INNOAQUA project development. New reports including these updates will be submitted by M30 (D4.5) and M48 (D4.6).

References

1. (FAO), Food and Agriculture Organization of the United Nations. *The State of World Fisheries and Aquaculture 2020*. : Sustainability in action, 2020. .
2. European Green Deal. [Online] https://commission.europa.eu/strategy-and-policy/priorities-2019-2024/european-green-deal_en.
3. Green Deal Industrial Plan. [Online] https://commission.europa.eu/strategy-and-policy/priorities-2019-2024/european-green-deal/green-deal-industrial-plan_en.
4. Circular Economy Action Plan. [Online] https://environment.ec.europa.eu/strategy/circular-economy-action-plan_en.
5. Green Deal and Packaging. [Online] https://ec.europa.eu/commission/presscorner/detail/en/ip_22_7155.
6. Ecodesign for Sustainable Products Regulation. [Online] https://commission.europa.eu/energy-climate-change-environment/standards-tools-and-labels/products-labelling-rules-and-requirements/sustainable-products/ecodesign-sustainable-products-regulation_en.
7. Farm to Fork Strategy. [Online] https://food.ec.europa.eu/horizontal-topics/farm-fork-strategy_en.
8. Green Claims Directive Proposal. [Online] https://environment.ec.europa.eu/topics/circular-economy/green-claims_en.
9. EU Taxonomy for Sustainable Activities. [Online] https://finance.ec.europa.eu/sustainable-finance/tools-and-standards/eu-taxonomy-sustainable-activities_en#:~:text=The%20EU%20taxonomy%20allows%20financial,can%20be%20considered%20environmentally%20sustainable.
10. EU Taxonomy Navigator. [Online] <https://ec.europa.eu/sustainable-finance-taxonomy/>.
11. Guide to promote circularity in food packaging. Inèdit for Cluster Food Service, Packaging Cluster, Agència de Residus de Catalunya. [Online] 2022.



https://media.timtul.com/media/web_packagingcluster/CircularPack_guia_entregable_completa___20220419073948.pdf.

12. Circulate. Packaging checklist. [Online]

<https://www.circulate8.com/sustainability/packaging-checklist>.

13. —. Product Labels. [Online] <https://www.circulate8.com/sustainability/product-labels>.

14. Design for Recycled Content Guide. [Online]

<https://sustainablepackaging.org/projects/design-for-recycled-content-guide/>.

15. CEFLEX. Circular Economy for Flexible Packaging. [Online] <https://ceflex.eu/resources/>.

16. The International EPD System. [Online] <https://environdec.com/home>.

17. PD Norge . [Online] <https://www.epd-norge.no/>.

18. EU Novel Food Status Catalogue. [Online] <https://ec.europa.eu/food/food-feed-portal/screen/novel-food-catalogue/search>.

19. Summary of the applications submitted within the meaning of Article 10(1) of Regulation (EU) 2015/2283. [Online] https://food.ec.europa.eu/safety/novel-food/authorisations/summary-applications-and-notifications_en.

20. Pork sausages: Ambiental declaration document. [Online]

<https://api.environdec.com/api/v1/EPDLibrary/Files/b5084e5a-4cf0-4ac4-5657-08da12da9675/Data..>

21. Impossible Foods Inc. Sausage made from plants Life Cycle Assessment. [Online] 2020.

<https://impossiblefoods.com/sustainable-food/sausage-life-cycle-assessment-2020>.

22. BERCA S.A. Estancia Ralicó. Argentine meat: Environmental Product Declaration. [Online]

2024. <https://api.environdec.com/api/v1/EPDLibrary/Files/529fd236-a71d-434e-5474-08dc2e3a662b/Data>.

23. Environmental Product Declaration of Coop Veal Meat. [Online] 2024.

<https://api.environdec.com/api/v1/EPDLibrary/Files/809ac77e-621c-4c03-ce39-08dc1e81dd8e/Data>.



24. Fileni. Environmental Product Declaration Petto di pollo biologico. [Online] 2021. <https://api.environdec.com/api/v1/EPDLibrary/Files/2152c83c-9b94-4e54-f525-08d93f6df637/Data>.
25. MultiX. Atlantic Salmon Environmental Product Declaration. [Online] 2023. <https://api.environdec.com/api/v1/EPDLibrary/Files/4ae4becd-d333-42fa-4800-08db1f315c5e/Data>.
26. Environmental Product Declaration. New Zealand King Salmon. [Online] 2021. <https://api.environdec.com/api/v1/EPDLibrary/Files/bc5ed8ef-4ed5-41b7-3a38-08d98fad225/Data>.
27. AquaChile. Environmental Product Declaration. Fish and Fish products (Salmonoids) . [Online] 2021. <https://api.environdec.com/api/v1/EPDLibrary/Files/ca298a24-e2bc-47e8-16eb-08d972a96257/Data>.
28. Spanish Government. Ministerio de Consumo. Nota sobre el empleo de las menciones "Sin Gluten" y "Sin lactosa" en el etiquetado, presentación y publicidad de los alimentos. [Online] https://www.aesan.gob.es/AECOSAN/docs/documentos/seguridad_alimentaria/gestion_riesgos/Menciones_sin_gluten_sin_lactosa.pdf.
29. Inalca. Dichiarazione Ambientale di Prodotto. Hamburger Surgelati. [Online] 2023. <https://api.environdec.com/api/v1/EPDLibrary/Files/1615b232-5ebe-42f9-9938-08db7e35bd3c/Data>.
30. Bran Cereale&Barilla. Gran Cereale granola croccante Dichiarazione Ambientale di Prodotto. [Online] 2023. <https://api.environdec.com/api/v1/EPDLibrary/Files/066937bc-6da8-4da8-27af-08db259f9365/Data>.
31. VOG products. Environmental Product Declaration. Apple Puree. [Online] 2020. <https://api.environdec.com/api/v1/EPDLibrary/Files/880bd242-e720-446c-96fe-08dc3c72202b/Data>.
32. Pan di Stelle &Barilla. Biscotto Pan di Stelle. Dichiarazione Ambientale di Prodotto. [Online] 2021. <https://api.environdec.com/api/v1/EPDLibrary/Files/9ed5c329-2f4f-4b8a-aa10-08d9c4927501/Data>.

33. Pavesi&Barilla. Ringo cacao, vaniglia, nocciola, cereali e cocco. Dichiarazione Ambientale di Prodotto. [Online] 2022. <https://api.environdec.com/api/v1/EPDLibrary/Files/8bb49a81-4c31-4997-cef5-08d9df0ea78f/Data>.
34. Mulino Bianco&Barilla. Fette Biscottate Dolcifette. Dichiarazione Ambientale di Prodotto. [Online] 2023. 2023 <https://api.environdec.com/api/v1/EPDLibrary/Files/b8ce8fbf-5ad8-43a0-dfdf-08dbf085b35e/Data>.
35. Wasa&Barilla. Wasa Multigrain, Surdeg Flerkorn. Environmental Product Declaration. [Online] 2021. <https://api.environdec.com/api/v1/EPDLibrary/Files/b691318e-cb62-42dd-27c5-08db259f9365/Data>.
36. Bauli. Buondì cioccolato. Environmental Product Declaration. [Online] 2023. <https://api.environdec.com/api/v1/EPDLibrary/Files/32a88b1f-abf7-4572-4f0f-08dbdfa90a02/Data>.
37. Conserve Italia. Soft Drink Multifruit. Environmental Product Declaration. [Online] 2023. <https://api.environdec.com/api/v1/EPDLibrary/Files/1c3aa00d-8b26-43ed-9d5d-08db7e35bd3c/Data>.
38. VOG products. Cloudy apple juice NFC. Environmental Product Declaration. [Online] 2020. <https://api.environdec.com/api/v1/EPDLibrary/Files/d456f870-eb2e-416c-96ff-08dc3c72202b/Data>.
39. OraSí. Environmental Declaration of the product plant-based rice beverage with vitamins and calcium. [Online] 2021. <https://api.environdec.com/api/v1/EPDLibrary/Files/435e9996-1537-48b8-a7c5-08d937ede007/Data>.
40. Barilla. Environmental Product Declaration. Pesto al Basilico 100% Vegetale. [Online] 2022. <https://api.environdec.com/api/v1/EPDLibrary/Files/c5680991-f4dd-40ee-10c5-08dacbb1d2f6/Data>.
41. AMB. High barrier Mono PET film for packaging applications : Environmental Product Declaration. [Online] 2022. <https://api.environdec.com/api/v1/EPDLibrary/Files/40770901-ae54-4f44-a74e-08da599e304a/Data>.

42. —. Multilayer PET/PE/EVOH/PE peel film for packaging applications: Environmental Product Declaration. [Online] 2022.
<https://api.environdec.com/api/v1/EPDLibrary/Files/774e5175-019e-4977-a74f-08da599e304a/Data>.
43. SEE. Environmental Product Declaration For Cell-Aire® Polyethylene Foam Packaging 1mm. [Online] 2023. <https://api.environdec.com/api/v1/EPDLibrary/Files/1c7e8b6c-bb41-4352-91a4-08dc0c71a3ba/Data>.
44. Basi. BASIFLEX Environmental Product Declaration. [Online] 2023.
<https://api.environdec.com/api/v1/EPDLibrary/Files/6468e327-4742-498c-28fd-08db259f9365/Data>.
45. Vasetti in polistirene bianco per 125g di yogurt. Dichiarazione Ambientale di Prodotto. [Online] 2020. <https://api.environdec.com/api/v1/EPDLibrary/Files/8f337be9-8379-4320-981d-8f535c81b1ee/Data>.
46. *Microalgae of the genus Nannochloropsis: Chemical composition and functional implications for human nutrition*. L. Zanella, F. Vianello. 2020, Journal of Functional Foods, Vol. 68, p. 103919.
47. *Biomass nutrient profiles of the microalga Phaeodactylum Tricornutum*. M.M. Reboloso-Fuentes, A. Navarro-Pérez, J.J. Ramos-Miras, J.L. Guil-Guerrero. 1, 2001, Journal of Food Biochemistry, Vol. 25, pp. 57-76.
48. Deoleo. Environmental product declaration for extra virgin olive oil. [Online] 2023.
<https://api.environdec.com/api/v1/EPDLibrary/Files/d7e05ffc-fb2d-41ca-265c-08db259f9365/Data>.
49. Aker biomarine. Environmental Product Declaration QRILL. [Online] 2023.
<https://api.environdec.com/api/v1/EPDLibrary/Files/b04f3195-7403-4fcb-b021-08db681d8c95/Data>.
50. Isanatur, Contactica, Zade, Biozoon, Amerex, Indukern. PCR Under development – Functional food ingredients. [Online] <https://www.environdec.com/pcr-library> Search for functional food ingredients.



51. Pereira, L. A Review of the Nutrient Composition of Selected Edible Seaweeds. [ed.] Vitor H. Pomin. *Seaweed: Ecology, Nutrient Composition and Medicinal Uses*. s.l. : Nova Science Publishers, 2011, pp. 15-47.
52. *Agar content of estuarine seaweed Gracilaria using different cultivation methods*. M. Mendes, Du. Fortunato, J.Cotas, D. Pacheco, T. Morais, L. Pereira. 2, 2022, Applied Food Research, Vol. 2, p. 100209.
53. *Atlantic algae as food and their extracts*. . 2023;1:15–31. Pereira., L. 2023, Explor Foods Foodomics, Vol. 1, pp. 15-31.
54. TCI SANMAR CHEMICALS S.A.E. PVC Suspension resin : Environmental product declaration. [Online] 2022. <https://api.environdec.com/api/v1/EPDLibrary/Files/3847f9cc-385e-4495-9b4b-08da3d5507cb/Data>.
55. Novapet SA. Environmental product declaration: PET resin. [Online] 2023. <https://api.environdec.com/api/v1/EPDLibrary/Files/900ba84b-3d42-45fc-aaf9-08db4497f421/Data>.
56. Hellenic petroleum. Environmental product declaration: Ecolen Polypropylene homopolymer resins. [Online] 2023. <https://api.environdec.com/api/v1/EPDLibrary/Files/eb47e6c7-329e-46cf-1136-08dbca69748b/Data>.