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D3.1 – Characterisation results of algae biomass and fish/seafood processing waste

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List of Acronyms

Abbreviation / Acronym	Description
AA's	Amino acids
ALA	α-linoleic Acid
BCA	Bicinchoninic Acid Assay
BSA	Bovine serum albumin
DHA	Docosahexaenoic Acid
DEMO#1	Integration salmon and microalgae cultivation in a coupled RAS-IMTA in WP2
DEMO#2	Integration sole and seaweed cultivation in a coupled RAS-IMTA system in WP2
DW	Dried Weight
EC	European Commission
EPA	Eicosapentaenoic Acid
EU	European Union
FPH's	Fish Protein Hydrolysates
FPS	Fish Processing Side Streams
FW	Fresh Weight
IMTA	Integrated Multitrophic Aquaculture
M	Month
MUFA's	Monounsaturated Fatty Acids
ND	Not detected
PUFA's	Polyunsaturated Fatty Acids
RAS	Recirculating Aquaculture Systems
SAFA	Saturated Fatty Acid
T	Task
WP	Work Package

Executive Summary

This document, Deliverable 3.1, is part of the Task 3.1 of the INNOAQUA project and describes the composition of algae biomass (microalgae, macroalgae) and fish by-products (non-compliant surimi sticks, salmon heads, salmon skins), which will be valorised, through extraction processes, to obtain ingredients that meet food formulators requirements (Task 3.2, Task 3.3, Task 3.4).

The proximate analysis for the major constituents (moisture, ash, lipid, protein contents) is given. In addition, fatty acids, B-vitamins content, carbohydrate and carotenoids are given for microalgae; carbohydrates (calculated) are given for macroalgae; and carbohydrates (calculated), amino acids, elements, heavy metals and microbiology are given for fish by-products. The composition is taken into account to select the best biomass for the production of the following ingredients:

- From microalgae: PUFA's and carotenoid rich oil, proteins, hydrophilic vitamins, peptides and amino acids, and residual polysaccharides
- From macroalgae: proteins, agar and residual mix of polysaccharides and proteins.
- From fish by-products (non-compliant surimi sticks, salmon heads and skins): proteins.

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 (FAO, 2020), 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 main objective of the deliverable D3.1 *Characterisation results of algae biomass and fish/seafood processing waste* is to provide the biomasses composition and select the most promising ones for further processing into valuable extracts in the subsequent tasks. So, the results of the composition will be used for the selection of the biomass to develop food ingredients in tasks T3.2, T3.3 and T3.4 for the development of seafood products in WP4.

For each biomass, this documents details:

i) General description of the biomass

ii) Biomass preparation and preservation

iii) Analytical methods used and results obtained

iv) Criteria for biomass selection and selected biomasses

Prior to the development of this task, the characterisation parameters have been discussed and defined with all the WP3 partners. This document together with the expected extraction yields also aims to serve as a first approach to the calculation of the quantities of each ingredient and seafood product that will be produced within the INNOAQUA project, which is essential to calculate the necessary amount of biomass to be produced in WP2.

This work, which was developed under Task 3.1 *Characterisation of algae biomass and fish by-products*, started in M6 and finished at M18. The composition of the new biomass batches generated after the completion of Task 3.1 will be reported in the corresponding tasks and deliverables: D3.2 (microalgae; T3.2), D.3. (macroalgae; T3.3) and D3.4 (fish by-products; T3.4) (M36). These new batches of biomass will be used for the production of ingredients.

1.2 Structure of the document

This document is divided into 4 sections:

Section 1 serves as an Introduction.

Section 2 summarises the Methodology that has been employed to analyse the biomass.

Section 3 includes the Results of the biomass characterisations within the INNOAQUA project.

Section 4 provides the Conclusions, including the selection of the target biomass.

1.3 Relation to other project deliverables

As mentioned above, the present document D3.1 *Characterisation results of algae biomass and fish/seafood processing waste* describes the composition of the biomass which will be taken into account to develop and optimize the algae and fish by-products processing techniques in the subsequent tasks in order to obtain the ingredients detailed above:

- From microalgae, biomass produced by NORCE, first batches in Task 2.2, large batches in Task 2.4. Microalgae will be processed in Task 3.2 to produce the following ingredients: PUFA's and carotenoid rich oil, proteins, hydrophilic vitamins, peptides and amino acids and the residual fraction (to be further processes into plastic resin)
- From macroalgae, biomass produced by A4F, first batches in Task 2.3, large batches in Task 2.4. Macroalgae will be processed in Task 3.3 to produce the following ingredients: proteins, agar and the residual fraction (to be further processes into plastic resin)

- From fish by-products: non-compliant surimi sticks generated by PESCANOVA, salmon heads and skins generated by VIKING AQUA. Fish by-products will be processed in Task 3.4 to produce functional Fish Protein Hydrolysates (FPH).

So, WP2 (NORCE and A4F) is providing feedback to WP3 regarding the biomass production (Task 2.4; Milestone 4). In WP3, the different biomass compositions have been used to select the specific biomasses (Task 3.1) and are being used to calculate the extraction yields to optimise the processing techniques from microalgae by ALGEMY (Task 3.2, D3.2, (M36)), macroalgae by A4F (Task 3.3, D3.3 (M36)), and fish by-products by LEITAT (Task 3.4, D3.4, (M36)). WP3 (ALGEMY, A4F and LEITAT) is providing feedback to WP4 regarding the biomass composition and the estimated extraction yields, mainly to Task 4.1 for the calculation of the required biomass amounts for the expected seafood production (D4.1 *Report on product prototypes specifications* (M12) and updates D4.5 (M30) and D4.6 (M48)). These values will be taken as a first estimation and not as a final value. WP3 will also provide feedback to WP4 regarding the obtained extracts, which will be converted into ingredients in WP4 (task 4.2) and assessed in WP5 (SUSTAINN and PERSEUS BVBA) regarding sustainability (D5.1, D5.2, D5.5, D5.6, D5.7 and D5.8).

2. Methodology

In this section, a detailed description for each of the INNOAQUA. biomass used is given. The biomasses are divided into subsections. A short description of the biomass, preparation and preservation, and analytical methods are provided. Table 1 indicated the parameters analysed.

Table 1 Parameters analysed in INNOAQUA biomasses

Biomass	Parameter
Microalgae	moisture, ash, total lipids, protein, total fatty acids content and profile, B-vitamins content and profile, carbohydrates and total carotenoids content and profile
Macroalgae	moisture, ash, total lipids, protein, carbohydrates (calculated)
Fish by-products	moisture, ash, total lipids, protein, carbohydrates (calculated), amino acids, macroelements, microelements, heavy metals, microbiology

2.1 Microalgae

2.1.1 General description of the biomass

A small batch of two strains of three different microalgae species (six strains in total), were produced in DEMO#1 and processed as part of WP2: *Chlorella sorokiniana (UTEX 1230 and UTEX 2805)*, *Phaeodactylum tricornutum* (UTEX 640 and B58 - a Norwegian local strain) and *Nannochloropsis oculata (UTEX 2164 and NIVA-3/04)*. The reason to choose three different microalgae sources was that they differ in terms of composition. *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 being the omega-3 EPA and DHA. *Nannochloropsis oculata* is especially relevant for the production of PUFA's, as it can produce not only large amounts of EPA, but also carotenoids. By processing the three biomasses separately, it is expected to obtain high quality ingredients, containing an excellent and complementary nutritional profile.

For each of the six different strains, an initial first batch of ca. 25g (DW) was provided by WP2, which was used exclusively to perform the first characterisation reported here. These results served to select the best strain for each specie (three in total) to be produced in larger amounts for their processing as part of T3.2.

2.1.2 Biomass preparation and preservation

The biomass was cultivated by NORCE in Bergen, Norway, in 25L L-Gem photobioreactors, and partially characterised there (fatty acids profile and moisture). The remaining biomass was frozen and shipped to Spain. ALGEMY determined the ash content, carotenoids profile, B vitamins profile, total lipids content and carbohydrates content, while LEITAT quantified the protein. The biomass was kept frozen until immediately before each characterisation, except for some of the determinations (total lipid content, carotenoids profile and total carbohydrate content), in which lyophilised biomass was used. The biomass lyophilization was performed in a Telstar CRYODOS-50 instrument operating at 0.74 mBar and -32ºC.

2.1.3 Analytical methods

Dry matter

The dry matter content was determined by gravimetric loss of free water by heating to 95ºC overnight. Samples were analysed in triplicate (n=3).

Ash content

Total ash was determined by gravimetry by calcination in a muffle furnace. The samples were weighted in a porcelain crucible and placed in a muffle at 580ºC overnight. Due to a lack of sample, the determinations were performed with the big batch of biomass produced lately, and thus, only performed for the previously selected strains. For the rest of the strains, the ash content was calculated as the difference of all the other compounds in DW. Samples were analysed with a single replicate, in a well stablished and verified analytical method.

Protein content

Total protein was obtained from the total nitrogen determination, using a conversion factor of 6.25. Total nitrogen was determined by the Kjeldahl method: digesting the sample in sulphuric acid with a catalyst and then distilling and titrating the generated ammonia with 1M HCl. Samples were analysed in triplicates (n=3).

Total lipids content and profile of fatty acids

The fatty acids profile was determined by gas chromatography using a flame ionization detector (GC-FID). Fatty acids were extracted and derivatized to fatty acid methyl esters (FAME) by direct esterification (2M HCl in methanol, 90 °C for 2 h) and then extracted with iso-octane. The total fatty acids content is calculated from the sum of all the individual fatty acids. Samples were analysed in duplicates (n=2).

Total lipid content

The total lipid content was determined with a gravimetric method, using a modified Folch's extraction (Folch J., 1957). Samples were analysed in triplicates (n=3).

Total B-vitamins content and profile

The profile of vitamins from the B group was quantified using a LC-MS/MS method. The total content of B-vitamins was calculated from the sum of the individual content of each vitamin. Due to a lack of sample, the determinations were performed with the big batch of biomass produced lately, and thus, only performed for the previously selected strains. Samples were analysed with a single replicate, in a well stablished and verified analytical method.

Total carbohydrate content

The total carbohydrate content of the samples was determined following the phenol-sulphuric acid colorimetric method, adapted for algae biomass (W. Chen, 2023). Samples were analysed in triplicates (n=3).

Total carotenoids content and profile

The carotenoids profile was determined with a HPLC-DAD method, after an extraction with ethanol-hexane-water (M.C. Cerón-García, 2018). The total carotenoids content was calculated as the sum of all the individual carotenoids. Samples were analysed with a single replicate, in a well stablished and verified analytical method.

2.2 Macroalgae

2.2.1 General description of the biomass

Two different macroalgae species were produced and processed as part of WP2, during this reporting period, each one from a different macroalgae genus: *Gracilaria* and *Ulva*. A third genus, *Porphyra*, is also a target species but not thought as the main source of protein in the project. On the other hand, for practical reasons related with its life cycle, it will be produced only during 2025. The reason to choose three different macroalgae genus was the different composition of each of them. *Gracilaria* is known for its agar production, as well as the possibility to have high protein content. *Porphyra* and *Ulva* have a high protein content. In all the cases, these are commercially known species, all approved to be used as food by the EU legislation. Several batches of *Ulva* and *Gracilaria* were produced during this stage of the

project. All were analysed primarily for protein content and the batches with best results were fully characterised.

2.2.2 Biomass preparation and preservation

At this stage of the project, the work includes only results for the two main species to be used, *Ulva* and *Gracilaria*. The biomass was produced by A4F in the context of WP2. In the absence of a functional DEMO#2 site, the partner had an alternative production site that used the same free-floating technology. Attention was algo given to the characteristics of the water, especially in terms of nutrients, to make it similar to future conditions. All the biomass was produced in open bioreactors, in free-floating conditions and in flowthrough, outdoors, and therefore subjected to the natural environmental conditions.

Upon harvesting from the bioreactors, biomass was briefly washed with freshwater and dried, indoors, in a ventilated drying room, at a maximum temperature of 40ºC. All the biomass was then placed in multilayer bags (paper and plastic), closed and kept in a cool and dark environment until the analysis. These conditions are actually the standard packaging and storage conditions used by partner A4F for all industrial macroalgae processes.

For analytical methods the macroalgae batches were cut using a mixer grinder and then sieved for a uniform particle size.

2.2.3 Analytical methods

Dry matter

The dry matter content was determined by gravimetric loss of free water by heating to 105ºC for a period of 5 hours. Samples were analysed in duplicate (n=2).

Ash content

Total ash was determined by gravimetry by calcination in a muffle furnace. The samples were weighted in a porcelain capsule and placed in a muffle at 580ºC overnight. Samples were analysed in duplicate (n=2).

Protein content

Protein quantification for complete macroalgae characterisation involves cell disruption to release proteins into the aqueous medium for measurement. To achieve this, the fine macroalgae powder, previously sieved to 400 µm, must be diluted with distilled water and vortexed manually for about 20 minutes. Protein quantification was performed using a colorimetric Bicinchoninic Acid Assay (BCA), which measures protein concentration based on the colour change produced by the reaction between proteins and the working reagent (50A :1B), with absorbance read at 562 nm, using a bovine serum albumin (BSA) standard curve for calibration. After determining the dilution factor and measured the absorbance values, the protein concentration in g/L is determined using the following equation: Protein(g/L) = Abs/m * Dilution Factor * 10^{-3}, where m is the sample mass. Finally, the protein content could also be determined in terms of dry weight and expressed as a percentage (%). Samples were analysed in duplicate (n=2). (Shen, 2023)

Fat content

The lipid quantification method used in this work was a solvent extraction-based technique adapted from Ryckebosch, Muylaert, and Foubert, but now optimized for extracting total and non-polar lipids from macroalgae. Samples were analysed in duplicate (n=2). (Ryckebosch, Muylaert, & Foubert., 2012)

Carbohydrate content

Carbohydrates were estimated as the difference between protein, fats and ash, on DW basis.

2.3 Fish by-products

2.3.1 General description of the biomass

Fish Processing Side Streams (FPS) were collected as described below.

Non-compliant surimi sticks were provided by PESCANOVA. Surimi sticks are analogues of high-priced products such as shrimps, scallops, lobsters, elvers and crab legs. They are made of fish flesh that has been deboned, then minced into a paste and frozen as blocks with sorbitol

and/or phosphates as stabilisers. Finally, blocks are mixed with other ingredients, namely salt, starch, water and oil, and then jellified in its final shape to be commercialised. The wastes are cuts of the final product (surimi sticks) caused by line breakdowns. Thus, it is a jellified mass of difficult handling, even as for animal feed, which cannot be easily reutilized due to its consistency. This waste can range 5% to 8% of the total surimi seafood production (Pescanova) and it was referred in INNOAQUA as "non-compliant surimi sticks". This by-product was delivered to LEITAT as frozen sample to ensure the preservation during transport and storage.

Salmon heads and skins were provided by VIKING AQUA (VA RAS). Salmon smolts are typically transferred to the sea after spending between 8 to 18 months in freshwater, where they grow from fry to smolt stage and reach a weight of around 100 grams. At this stage, the smolts are ready to adapt to seawater and are therefore moved to sea-based aquaculture facilities where they continue to grow until they reach slaughter weight. After being transferred to the sea, it takes an additional 14-18 months before the salmon reach an average slaughter weight of 4.7 kg and are ready for slaughtering. It's important to note that the exact age at sea transfer and slaughtering can vary somewhat depending on factors like feeding, growth conditions, and the specific practices of each aquaculture facility. A fish processing company imports fish from VA RAS. The fish is gutted and processed for packaging. This process yields bioproducts (gut, head-cut, backbone, belly, off-cut, etc), which can find established or un-stablished markets.

Heads represent 12 % of the whole salmon. Skins are normally part the final trim (off-cut), which represents a 5%. They can be separated if the fish is of low quality, but this is season dependent. Skin removal is not always feasible, and the composition is more variable than for heads. The highest price factories can get for these products are when used directly for human consumption. This is not always possible because market is getting saturated. Pet food producers hydrolyse this by-product to produce fish oil.

2.3.2 Biomass preparation and preservation

At LEITAT, non-compliant surimi sticks were defrosted, and ground into a paste using Retsch Knife Mill GRINDOMIX GM 200 to ensure uniform biomass and small particle size. They were preserved at -20ºC until the analysis.

At LEITAT, salmon heads and skins were defrosted, minced using a knife and ground into a paste using Retsch Knife Mill GRINDOMIX GM 200 to ensure uniform biomass and small particle size. They were preserved at -20ºC until the analysis.

2.3.3 Analytical methods

Dry matter and moisture

The dry matter content was determined by gravimetric loss of free water by heating to 105ºC for a period of 5 hours. Samples were analysed in triplicate (n=3).

Ash content

Total ash was determined by gravimetry by calcination in a muffle furnace. The samples were weighted in a porcelain capsule and placed in a muffle at 550ºC for 2 hours. Samples were analysed in triplicate (n=3).

Protein content

Total protein was obtained from the total nitrogen determination, using a conversion factor of 6.25. Total nitrogen was determined by the Kjeldahl method: digesting the sample in sulphuric acid with a catalyst and then distilling and titrating the generated ammonia with 1M HCl. Samples were analysed in triplicates (n=3).

Amino acids content

Total Amino acids content was analysed on defatted sample (except for surimi) after acid and alkaline digestion with 6M HCl and 4M NaOH for 22 h at 110°C, respectively. The determination was performed by a high-resolution liquid chromatography (HPLC-DAD) Amino acids were derivatized with OPA / FMOC and quantified using sarcosine as internal standard. Samples were analysed in duplicates (n=2).

Fat content

The fat content was determined with a gravimetric method, using a modified Soxhlet method (AOAC 948.15). The sample was dried at 65ºC for 48h and weighed within a Soxhlet extractor with petroleum ether. Samples were analysed in triplicate (n=3).

Carbohydrate content

Carbohydrates were estimated as the difference between protein, fats and ash, on DW basis.

Elements and heavy metals

The sample was digested with a mixture of ultrapure concentrated nitric acid in the analytical microwave (Microwave Anton Paar Multiwave 7000) at 250ºC. The obtained digestion residue was properly diluted in order to analyse the elements of interest by ICP-MS (Triple quadrupole ICP-MS Agilent 8900 ICP-QQQ). The quantification was performed by interpolation in a calibration curve prepared with commercial standards of the elements of interest. Samples were analysed in triplicate (n=3).

Microbiology

The following methods were used for the analysis of the microorganisms:

-Rapid detection method *Salmonella*. Rapid *Salmonella*. According to UNE EN ISO 16140.

-Method for selective counting of *Escherichia coli*. 3M Petrifilm EC. Validated according to UNE EN ISO 16140.

-ISO 4833-2:2014 Colony count at 30°C by surface plating techniques.

-ISO 21527:2008 Horizontal method for the enumeration of yeast and moulds.

3. Results

3.1 Microalgae

The six microalgae strains were cultivated and harvested in Norway by NORCE. Figure 1 shows the appearance of the six strains once harvested. For most of the characterisations, the microalgae biomass were used in paste form, as in Figure 1, but for the total carbohydrates,

total lipids and carotenoid profile they were used after lyophilization (

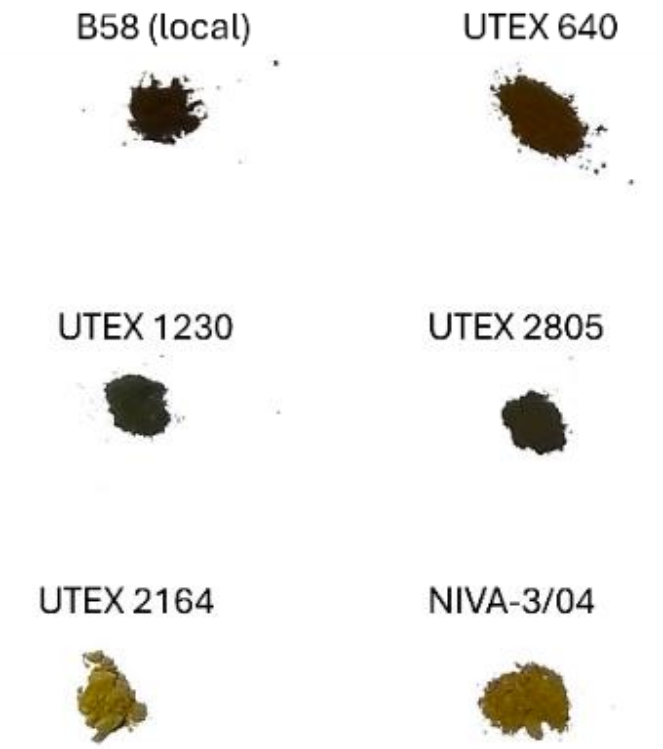

Figure 2).

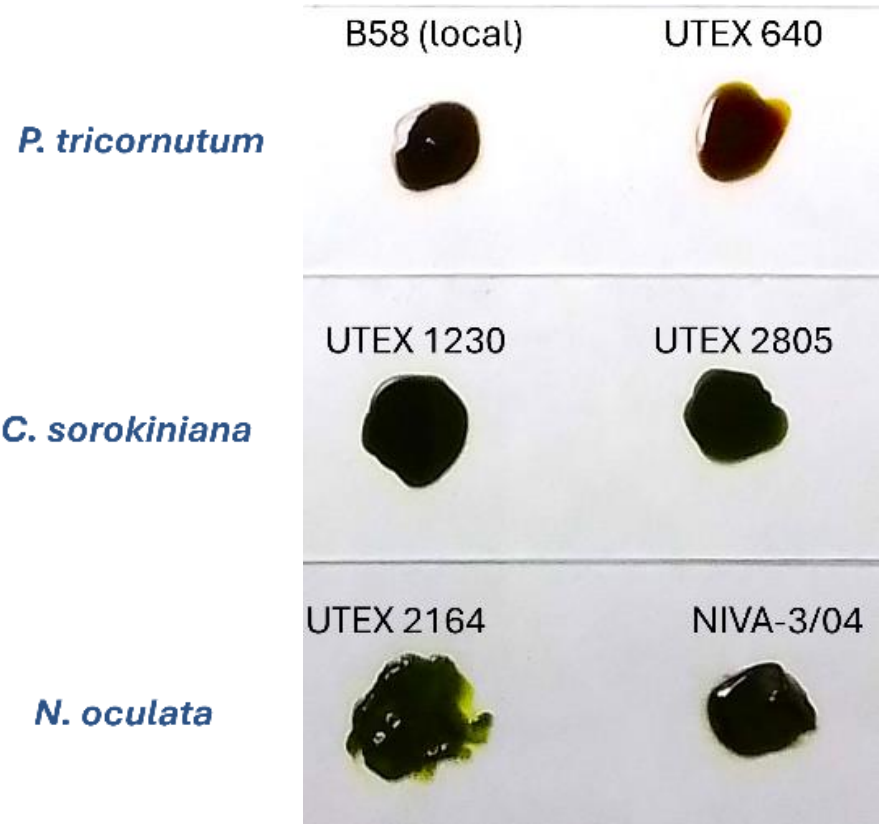

Figure 1. Appearance of the six microalgae strains once harvested

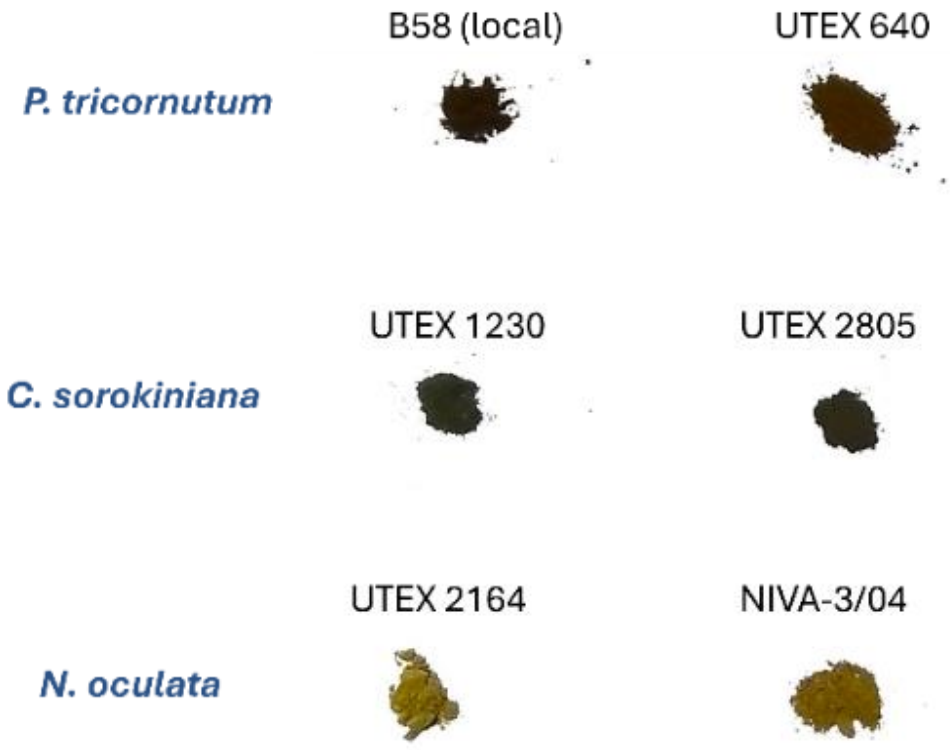

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Figure 2. Appearance of the six microalgae strains after lyophilization

The six microalgae strains were characterised as described in Section 2.

Table 2 and Figure 3 show the main values obtained.

Table 2. Characterisation of microalgae

	P. tricornutum		*C. sorokiniana*		*N. oculata*	
Parameter (% DW)	**B58 (local)**	**UTEX 640**	**UTEX 1230**	**UTEX 2805**	**UTEX 2164**	**NIVA-3/04**
Dry matter	22.9±0.0	25.0±0.0	21.1±0.1	26.3±0.1	22.2±0.3	22.0±1.5
Ash	14.8 (*)	-	5.9 (*)	-	-	(**)
Total lipids	18.1±0.1	18.6±0.6	16.3±0.6	17.7±0.1	29.0±0.1	22.5±0.6
Proteins	31.0±0.1	34.8±1.4	42.9±2.0	33.7±1.9	35.9±2.2	36.4±0.4
Total carbohydrates	14.7±0.7	29.5±2.2	32.6±1.7	43.0±2.6	13.9±2.1	14.5±2.2
Total carotenoids	0.55	0.11	0.97	0.49	0.42	0.33
Total B-vitamins	0.010 (*)	-	0.024 (*)	-	-	(**)

(*) Due to lack of sample, these parameters were measured from the 2nd biomass batch, and thus, are only available for the selected strains. Results cans slightly vary from one batch to another.
(**) Results not yet available. The results will be included in the Deliverable 3.2

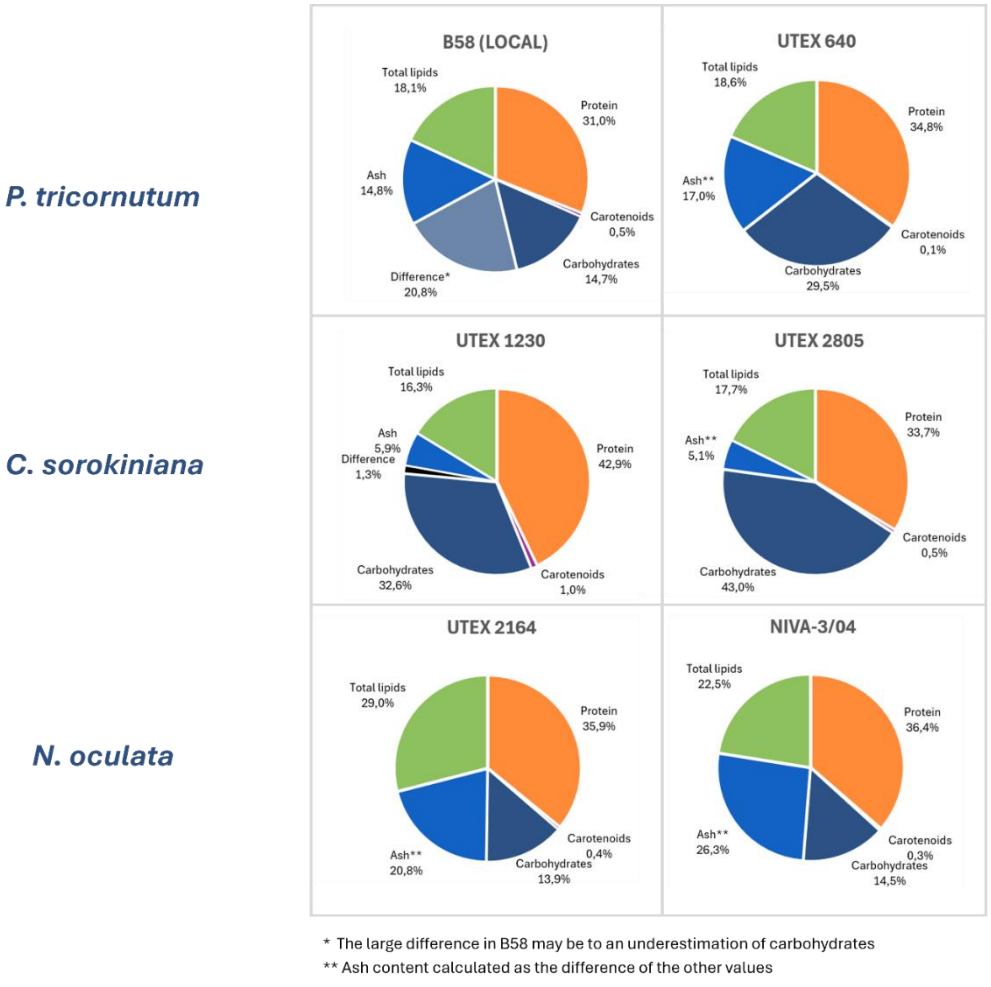

Figure 3. Composition of the different microalgae strains

It is important to note that for some species, both strains present similar results, while for others, there are important differences among the two strains. In this sense, the composition of both *N. oculata* strains is similar, while for *C. sorokiniana*, the UTEX 1230 strain has a much larger protein content than UTEX2805, while the last produces more carbohydrates. For the *Phaeodactylum* strains, the protein content is similar for both, but important differences in the carbohydrates and carotenoids production were found. Regarding the strain B58, it is important to note that the value obtained from the sum of the DW content of each individual component does not reach the 100%, and the error is quite large. An underestimation of the carbohydrates content due to an uncomplete biomass digestion is the main hypothesis to explain this result, and if it was the case, the carbohydrate content of both *P. tricornutum* strains would be similar. Due to lack of more sample, it was not possible to repeat the measurement yet. It will be re-analysed during the following weeks, and the results will be included in D3.2, together with the overall characterisation of the B58 strain produced in the bigger batch.

It is also interesting to compare the results obtained from the different species. *N. oculata* strains produce more lipids, while *C. sorokiniana* strains produce more carbohydrates and it has a considerably lower amount of ashes. A possible explanation for this reduced amount of ashes compared to the other species is that *C. sorokiniana* has been cultivated in freshwater, while *N. oculata* and *P. tricornutum* have been cultivated in sea water and brackish water, respectively, and thus, the biomass may contain a larger amount of salts.

To determine the primary constituents of each fraction, a more thorough study was conducted for the lipids, carotenoids and vitamins. Regarding the lipids, a determination of the fatty acids profile present in the sample was performed by gas chromatography. Table 3 summarizes the results obtained, while Figure 4 shows a graphic representation of the content of fatty acids grouped into saturated fatty acids (SAFA's), monounsaturated fatty acids (MUFA's), polyunsaturated fatty acids (FUPA's) and others/unknown (all the non-identified fatty acids).

Table 3. Fatty acids profile of the microalgae strains

	P. tricornutum		*C. sorokiniana*		*N. oculata*	
Fatty acids (% DW)	**B58 (local)**	**UTEX 640**	**UTEX 1230**	**UTEX 2805**	**UTEX 2164**	**NIVA-3/04**
14:0	0.70±0.02	0.72±0.01	0.01±0.01	0.03±0.01	1.32±0.02	0.89±0.03
16:0	1.78±0.03	3.08±0.04	1.90±0.01	2.45±0.07	4.38±0.08	4.00±0.18
16:1 n-x	0.16±0.04	0.04±0.01	0.14±0.01	0.01±0.01	0.01±0.01	0.02±0.01
16:1 n-9	ND	ND	0.14±0.01	0.01±0.01	ND	0.01±0.01
16:1 n-7	3.17±0.09	4.96±0.06	ND	0.04±0.01	4.66±0.07	4.29±0.20
16:2 n-7	0.14±0.01	0.15±0.01	ND	ND	0.09±0.01	0.08±0.01
16:2 n-6	ND	ND	1.50±0.01	1.64±0.07	ND	ND
16:2 n-4	0.60±0.01	0.16±0.01	ND	ND	ND	ND
16:3 n-4	1.40±0.02	0.61±0.01	ND	ND	ND	ND
16:3 n-3	ND	ND	1.20±0.02	1.36±0.11	ND	ND
18:0	0.03±0.01	0.06±0.01	0.05±0.01	0.08±0.01	0.05±0.01	0.06±0.01
18:1 n-9	0.10±0.01	0.16±0.01	0.25±0.01	0.66±0.01	0.38±0.01	0.36±0.01
18:1 n-7	0.06±0.01	0.16±0.01	0.06±0.01	0.04±0.01	0.08±0.01	0.09±0.01
18:2 n-6	0.21±0.01	0.23±0.01	3.34±0.02	3.17±0.04	0.28±0.01	0.29±0.01
18:3 n-6	0.09±0.01	0.08±0.01	ND	0.01±0.01	0.07±0.01	0.07±0.01
18:3 n-3	0.02±0.01	0.04±0.01	1.78±0.01	1.92±0.01	ND	0.01±0.01
18:4 n-3	0.04±0.01	0.12±0.01	ND	ND	0.02±0.01	0.02±0.01
20:4 n-6	0.40±0.01	0.15±0.01	ND	ND	0.68±0.01	0.69±0.01
20:5 n-3	3.10±0.08	3.27±0.03	ND	ND	4.16±0.01	4.80±0.19
24:0	0.21±0.01	0.39±0.01	0.02±0.01	0.02±0.01	ND	ND
24:1 n-9	0.07±0.01	0.08±0.01	ND	ND	ND	ND
22:6 n-3	0.12±0.01	0.35±0.01	ND	ND	ND	ND
other/unknown	1.17±0.03	0.66±0.02	0.82±0.01	0.91±0.02	0.90±0.02	1.02±0.07
PUFAs	6.12±0.12	5.15±0.07	7.82±0.06	8.09±0.15	5.30±0.01	5.95±0.22
MUFAs	3.56±0.14	5.40±0.07	0.52±0.01	1.02±0.02	5.14±0.09	4.77±0.23
SAFAs	2.72±0.06	4.24±0.04	1.99±0.01	2.58±0.08	5.75±0.10	4.95±0.22
Others/Unknown	1.17±0.03	0.66±0.02	0.82±0.01	0.91±0.02	0.90±0.02	1.02±0.07
Total Fatty Acids content	13.6±0.3	15.5±0.2	11.2±0.01	12.6±0.1	17.1±0.2	16.7±0.7

ND: Not detected

Among all the fatty acids, the polyunsaturated fatty acids are of major interest, and especially the omega-3 PUFA's (n-3), due to their health benefits. EPA (20:5(n-3)), DHA (22:6(n-3)) and ALA (18:3(n-3)) stand out. It is important to highlight that while *Chlorella* strains produce considerable amounts of APA but not EPA and DHA (as expected), *N. oculata* strains produce only EPA (the highest values), and *P. tricornutum* strains produce large amounts of EPA and some DHA. Regarding the overall FUPA's production, *C. sorokiniana* strains stand out as the

most productive, with similar values in both strains, and the local B58 stands out as the most productive among the *P. tricornutum* and *N. oculata*.

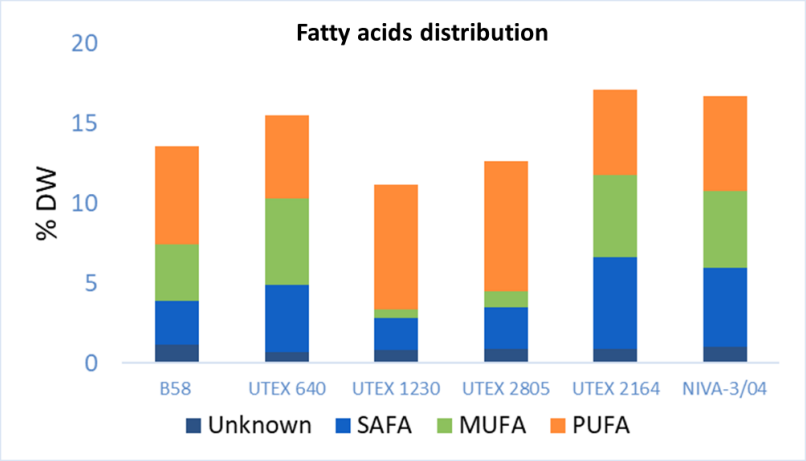

Figure 4. Fatty acids content of the six microalgae strains

For the carotenoids, the content profile was analysed by HPLC-DAD, and the results of the amount of each of the different compounds detected are summarized at Table 4.

Table 4. Microalgae carotenoids profile

	P. tricornutum		*C. sorokiniana*		*N. oculata*	
Carotenoids (% DW)	**B58 (local)**	**UTEX 640**	**UTEX 1230**	**UTEX 2805**	**UTEX 2164**	**NIVA-3/04**
But-Fucoxanthin	0.064	0.017	ND	ND	ND	ND
Fucoxanthin	0.153	0.016	ND	ND	ND	ND
Hex-Fucoxanthin	0.005	0.003	ND	ND	ND	ND
Diadinoxanthin	0.066	0.014	ND	ND	ND	ND
Diatoxanthin	0.205	0.048	ND	ND	ND	ND
Zeaxanthin	0.013	0.002	0.002	0.012	0.001	ND
Neoxanthin	ND	ND	0.077	0.001	0.063	ND
Violaxanthin	ND	ND	0.301	0.019	0.180	0.008
Canthaxanthin	ND	ND	0.278	0.017	ND	ND
Anteroxanthin	ND	ND	ND	ND	0.012	0.003
Vaucheroxanthin	ND	ND	ND	ND	0.013	0.001
Vaucheroxanthin ester	ND	ND	ND	ND	0,010	0.281
Lutein	ND	ND	0.037	0.261	ND	ND
Beta-carotene	0.04	0.006	0.271	0.183	0.139	0.035
Total carotenoids content	0.546	0.105	0.965	0.493	0.418	0.329

ND: not detected

For the carotenoid production, it is interesting to note how each different strain produces a different variety of compounds. Beta-carotene, a carotenoid that provides a natural green colour is common in all of them, even though the amount found in the *P. tricornutum* strains is much lower, as expected, as these diatoms are brown in colour. Other relevant carotenoids are Lutein and Zeaxanthin, both of which are antioxidants necessary for a good eye health. Among the INNOAQUA strains, only the *C. sorokiniana* strains produce Lutein, and in considerably different amounts, while most of the six strains produce low amounts of Zeaxanthin. On the other hand, Fucoxanthins are pigments typically found in brown seaweed and diatoms, thus only the *P. tricornutum* strains produce it. They possess antioxidant effect. Overall, and in the same way as for the FUPA's, the total amount of carotenoids produced is higher for the *Chlorella* strain UTEX 1230 and with the Local B58 strain also standing up.

For the B-vitamins profile, due to lack of sample, only the selected strains were analysed (from the big batch). Thus, only results for two strains (B58 and UTEX 1230) are available, while for NIVA-3/04 (last strain produced) results are still not available and will be included in Deliverable 3.2. Table 5 summarizes the results obtained.

Table 5. Microalgae B-vitamins profile

B-Vitamins (µg·100g^{-1} (DW))	*P. tricornutum* B58 (local)	*C. sorokiniana* UTEX 1230
B1	<424	976
B2	975	732
B3	7627	20488
B5	1695	1951
B6	<55	<63
B7 (Biotin)	26	22
B9	<106	<122
B12 (Cyanocobalamin)	<8	<10
Total B-vitamins	10323	24169

The results show that there are considerable differences among the two species, with *C. sorokiniana* producing two-fold the amount of B-vitamins than *P. tricornutum*. However, the values obtained are in general low, especially for vitamin B12 (N. Jalilian, 2019), which is the one with major interest, as most vegan diets present deficiencies in terms of B12 vitamin.

It is also important to note that the determination of the elements and metals content was decided to be performed later, once the biomass is treated and the different ingredients are obtained. It was agreed among the partners to not to consider these values for the strain selection, as they are less relevant than the proteins, carotenoids, lipids and PUFA's content. Thus, it was decided that these parameters will only be determined in the final ingredients to make sure that they are compliant with current regulations.

3.1.1 Microalgae strain selection

For the selection of one strain from each species, several factors were considered. The selection was not only based on the final composition of each strain, but also on the growth rate of each microalga. It is important to consider the growth rate of the culture, as it has a huge impact on the productivity of the different compounds. It is not only interesting to consider which compounds do each strain produces, but also how fast they are produced. As an example, if we consider two different microalgae, the first with a doubling time of 6 days and the second of 3 days, even if the protein content of the first was 30% higher than that of the second, the amount of protein produced after 10 days in the second culture would be higher than the first. For this reason, we decided to calculate a productivity index for the main compounds of interest, which were selected based on the relevance for the INNOAQUA project. The selected compounds were protein, PUFA's (and specifically EPA, DHA and ALA) and carotenoids (and specifically lutein and fucoxanthin). Carbohydrates were not considered relevant enough to be considered, as they are treated mostly as a residual fraction in the INNOAQUA project. For each of them, the **productivity index** was calculated from the ratio of the compound content (%, DW) in the biomass divided by the specific growth rate (μ) of the culture:

$$Productivity\ index\ for\ compound\ A = \frac{Content\ of\ A\ (\%,\ \ DW)}{\mu}$$

Table 6 shows the calculated productivity index of the selected compounds. Underlined is the highest productivity value among each specie, and in orange, the selected strain is marked.

Table 6. Microalgae productivity of the compounds of interest and strain selection

Productivity Index	*P. tricornutum*		*C. sorokiniana*		*N. oculata*	
	B58 (local)	UTEX 640	UTEX 1230	UTEX 2805	UTEX 2164	NIVA-3/04
Specific growth rate (μ)	0.64	0.43	0.87	0.73	0.55	0.56
Protein	19.82	14.84	37.48	24.52	19.85	20.29
PUFA's	3.90	2.22	6.82	5.89	2.93	3.29
EPA	1.98	1.40	-	-	2.30	2.67
DHA	0.08	0.15	-	-	-	-
APA	0.014	0.016	1.55	1.39	-	0.003
Carotenoids	0.35	0.04	0.84	0.36	0.23	0.18
Lutein	-	-	0.03	0.19	-	-
Fucoxanthin	0.14	0.01	-	-	-	-

Once the microalgae growth rate is considered, for both *P. tricornutum* and *C. sorokiniana* there is a strain that stands out among the pair, as it possesses a higher productivity index value in most of the categories. *P. tricornutum* B58 was selected due to its higher protein, PUFA's and carotenoids production rate, even though for some specific compounds such as DHA and APA, it has a slightly lower productivity index. In a similar way, *C. sorokiniana* UTEX 1230 was selected. In this case, the lutein productivity index of the selected strain is considerably lower than the other option, and thus, this supposes an important loss. However, the protein and PUFA's productivity indexes largely compensates for this decision. The difference in protein productivity among the *C. sorokiniana* strains was so large that choosing the other strain would have posed a problem to provide enough protein for the INNOAQUA project development. Regarding the selection of the *N. oculata* strain, the differences among the two options are narrow, especially in terms of the biomass final composition. However, once the growth rate was considered, one of them (NIVA-3/04) slightly stood out and was selected.

Thus, B58, UTEX 1230 and NIVA-3/04 are the strains that are being produced at larger scale in the INNOAQUA project.

3.2 Macroalgae

Figure 5 and Figure 6 show *Ulva* and *Gracilaria* biomasses, after being washed and dried.

Figure 5. Appearance of Ulva whole biomass, after being washed and dried - batch Ulva_20240314

Figure 6. Appearance of Gracilaria, whole biomass, washed and dried - batch Gracilaria_20240215

The macroalgae strains were characterised as described Section 2. Table 7 shows the values obtained.

Table 7. Characterisation of macroalgae

Parameter (% DW)	Ulva	Gracilaria
Dry weight	89.5 ± 0.2	91.6 ± 0.0
Ash	19.3 ± 1.6	19.5 ± 0.4
Fats	7.1 ± 0.3	4.7 ± 0.0
Proteins	11.4 ± 1.6	8.2 ± 0.5
Carbohydrates(*)	62.2	67.6

(*) carbohydrates were estimated as the difference between protein, fats and ash, on DW basis

Figure 7 shows the total characterisation of *Ulva*_20240314 on DW basis, as it was the batch with the highest protein content. Similarly, different *Gracilaria* batches were analysed for their composition, showing consistent results. Characterisation of batch *Gracilaria_20240215* is shown, in representation, in Figure 8. For *Ulva*, it is important to refer that the protein content is rather lower than expected, considering bibliographic references (10-32% protein DW) (Pereira, 2023). Very likely the method using is underestimating the total protein content, since it is more appropriate for soluble protein and, at this point, we have evidence that cell disruption needs to be improved. In that case, carbohydrates are being overestimated. All that

will be re-checked and validated in future WP3 deliverables. For *Gracilaria*, the direct quantification of agar is still missing, and it will be performed in the near future. In any case, the relationship between total carbohydrates and agar is well established, with at least 50% of that being agar. At this point, this characterisation is enough to validate a considerable presence of agar in this *Gracilaria* species (likely to be *Gracilaria gracilis*) and plan the biomass needs for upcoming tasks.

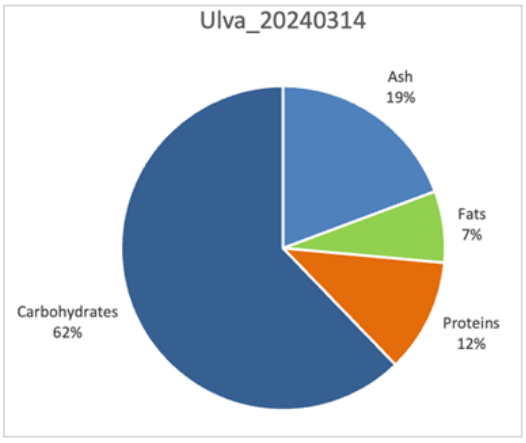

Figure 7. Characterisation of Ulva_20240314, on DW basis

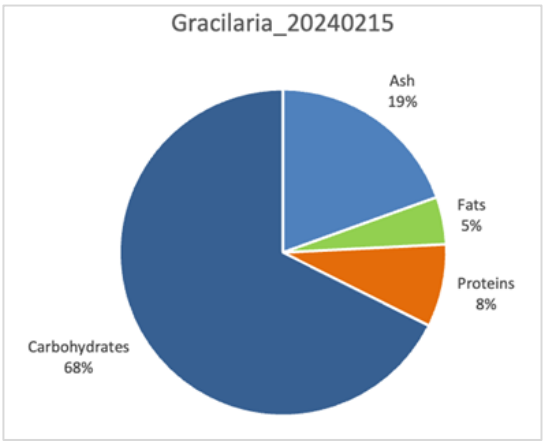

Figure 8. Characterisation of Gracilaria_20240215, on DW basis

3.2.1 Macroalgae strain selection

Considering current characterisation, the strains that originated the aforementioned batches for *Ulva* and *Gracilaria* were selected. Nevertheless, A4F will continue to screen and compare different strains, mainly of *Ulva*. Considering that *Ulva* is theoretically the species that could respond better to the presence of nutrients in the water, that its protein content may vary between strains, and that is relatively easy to screen and up-scale, A4F considers it is worth extending the effort of strain selection for that species. The main objective is to find a strain that is able to take better advantage of the nutrients levels expected at DEMO#2 and allows a better protein yield. Similar rational is used for *Gracilaria.* After validating that the species A4F selected *a priori* (likely *Gracilaria gracilis*) had enough agar content, different strains are not expected to yield significantly more agar, but rather to perform better in terms of growth and nutrient removal. In fact, in the case of *Gracilaria*, that nutrient removal performance is actually known to lead to more protein and less carbohydrates (hence less agar). In any case,

this continued strain screening for both species does not cause any constraints in the planned work, ingredient extractions, etc.

3.3 Fish by-products

Figure 9 shows the fish-by products before and after mincing and grinding into a paste.

Figure 9. Appearance of salmon skins, salmon heads and surimi sticks by-product.
From top to bottom: salmon skins, salmon heads and non-compliant surimi sticks. From left to right: entire samples, once minced and after grinding.

The fish-by products were characterised as described in Section 2. Table 8 shows the values obtained.

Table 8. Characterisation of fish by-products

Parameter (% DW)	Salmon skins	Salmon heads	Surimi sticks by-product
Dry matter	46.8±1.7	44.9±1.1	23.9±0.3
Ash	3.5±0.3	8.8±0.0	7.4±0.3
Fats	70.1±1.5	56.2±0.8	0.4±0.1
Proteins	30.7±4.6	22.5±0.7	27.7±0.4
Total amino acids (*)	74.5±4.0	49.2±0.5	33.5±1.8
Carbohydrates (**)	0.0	12.5 (***)	64.5

(*) total amino acids content was analysed on defatted sample except for surimi
(**) carbohydrates were estimated as the difference between protein, fats and ash, on DW basis
(***) in salmon heads, due to the heterogenicity of the sample, low values for protein, fats and ash were obtained and the difference as carbohydrates resulted overestimated.

Figure 10 shows the characterisation, on DW basis.

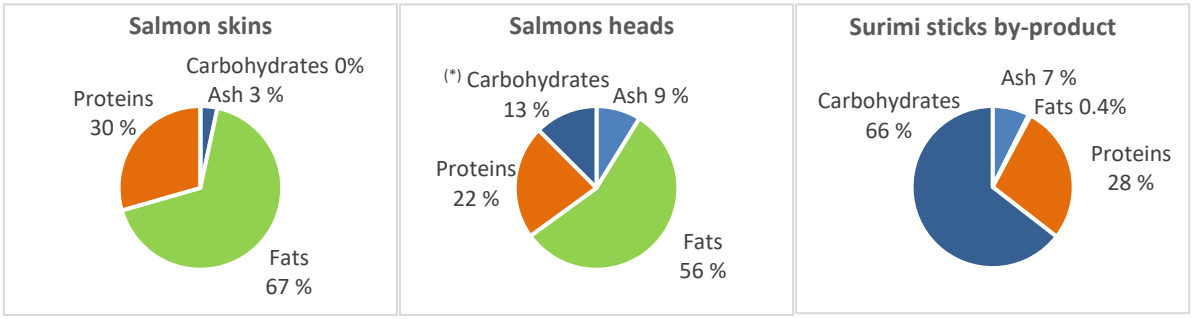

Figure 10. Characterisation of salmon skins, salmon heads and surimi sticks by-product, on DW basis

(*) in salmon heads, due to the heterogenicity of the sample, low values for protein, fats and ash were obtained and the difference as carbohydrates resulted overestimated.

From these results, it can be concluded that all fish by-products are rich in protein, which is the main compound of interest for the production of Fish Protein Hydrolysates. Protein content was 30% DW for skins, 22% DW for heads, and 28% DW for surimi. It is noteworthy that surimi has a high content of impurities, in particular carbohydrates (starch added in the

food production), which might reduce the protein purity in the FPHs. Impurities will be studied in Task 3.4.

Below, Table 9 and Figure 11 show the results of amino acids profile (AA's), on DW basis on defatted sample (salmon skins and heads) and on original sample (surimi). Surimi was not defatted for the analysis of amino acids because very little fat was present (0.4% fats) DW. Thus, results can be compared. Samples were analysed in triplicate (n=2).

Table 9. Total amino acids content of salmon skins, salmon heads and surimi sticks by-product

Parameter (mg / kg DW)	Salmon skins (defatted)	Salmon heads (defatted)	Surimi sticks by-product
ASP	6.7±0.2	4.8±0.1	3.8±0.1
GLU	11.3±0.2	7.4±0.4	6.7±0.3
SER	3.2±0.0	2.1±0.1	1.5±0.0
HYS	0.9±0.1	0.0±0.0	0.5±0.0
GLY	11.1±0.3	6.9±0.2	1.1±0.0
THR	2.8±0.1	1.7±0.1	1.6±0.1
ARG	6.4±0.2	3.4±0.1	2.3±0.2
ALA	5.7±0.1	3.5±0.1	2.0±0.1
TYR	1.7±0.2	0.8±0.0	1.2±0.1
CYS-CYS	0.00±0	0.0±0.0	0.2±0.1
VAL	3.4±0.2	2.2±0.1	1.7±0.1
MET	2.1±0.1	1.1±0.1	0.9±0.0
PHE	2.6±0.1	1.3±0.1	1.2±0.1
ILE	2.1±0.1	1.2±0.0	1.5±0.1
LEU	4.0±0.3	2.5±0.1	2.9±0.1
LYS	1.6±0.5	1.7±0.0	1.6±0.2
PRO	2.9±1.7	3.1±0.2	2.7±1.9
HYP	4.2±0.0	4.1±0.2	0.0±0.0
TRP	1.6±0.0	1.3±0.0	0.1±0.0
GLN	0.0±0.0	0.0±0.0	0.0±0.0
ASN	0.0±0.0	0.0±0.0	0.0±0.0
TOTAL	74.5±4.0	49.2±0.5	33.5±1.8

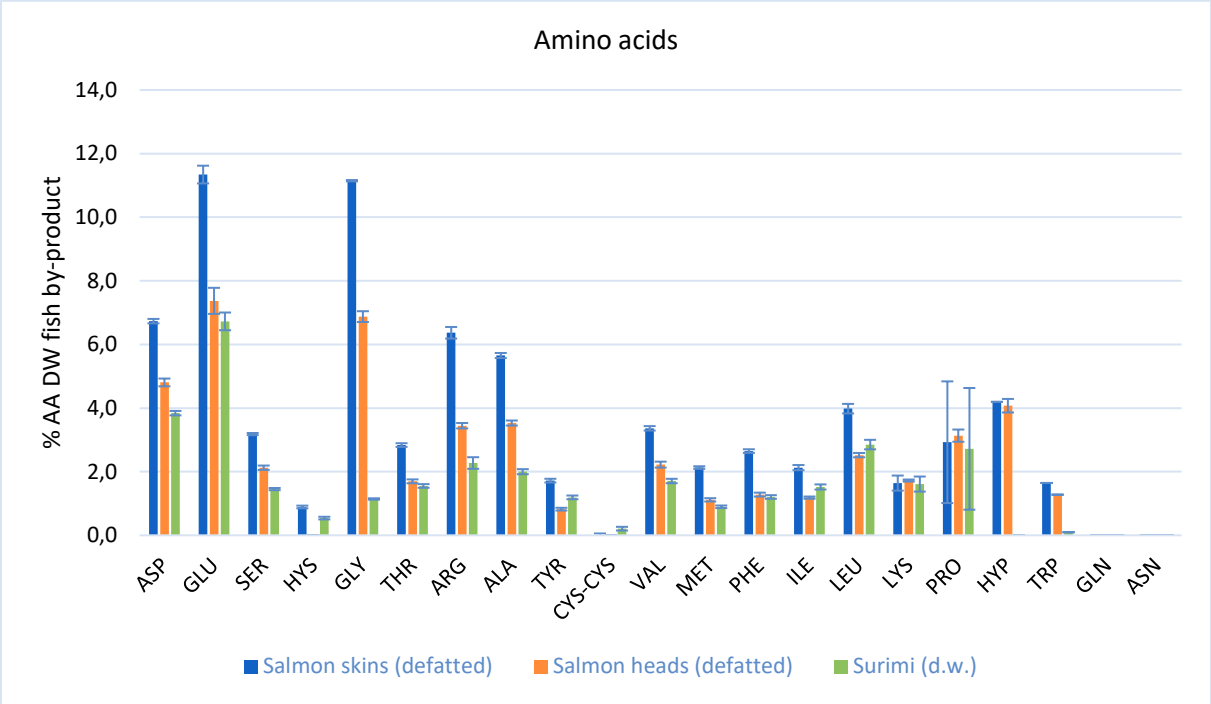

Figure 11. Total amino acids content of salmon skins, salmon heads and surimi sticks by-product. The values for salmon heads and skins were analysed on defatted samples, and they are comparable to those of surimi, which had a very low fat content.

The most abundant amino acids found in salmon skins and heads were glutamic acid, glycine, aspartic acid and arginine, which is consistent with data from the bibliography. In surimi the most abundant amino acids were glutamic acid, aspartic acid, leucine and proline. Total amino acids in salmon were higher (74.5 % DW for defatted skins and 49.2 % DW for defatted heads) than in surimi (33.5% DW surimi). It should be noted that here the amino acid content refers to defatted sample and therefore, the value is higher than the protein value when expressed on original sample in Figure 10.

Below, Table 10, Figure 12 and Figure 13 show the results of macroelements, microelements and heavy metals, on DW basis of fish-by product.

Table 10 Elements and heavy metals content of salmon skins, salmon heads and surimi sticks by-product

Parameter (mg / kg DW)	Salmon skins	Salmon heads	Surimi sticks by-product
Na	1529±176	4762±143	28211±1993
Mg	520±1	1156±94	642±78
P	7532±710	18334±509	1382±96
K	3649±344	4109±505	1376±69
Ca	6410±655	27564±2916	<420
Cr	<2.5	<2.5	<4.5
Mn	4±1	15±0	<4.5
Fe	13±0	15±4	<5
Cu	<2.5	<2.5	<4.5
Zn	16±1	78±7	<21
Se	<2.5	<2.5	<4.5
Mo	<2.5	<2.5	<4.5
I	<25	<25	<45
As	0.7±0.1	0.8±0.1	<0.5
Cd	<2.5	<2.5	<4.5
Hg	<0.5	<0.5	<0.5
Pb	<2.5	<2.5	<4.5

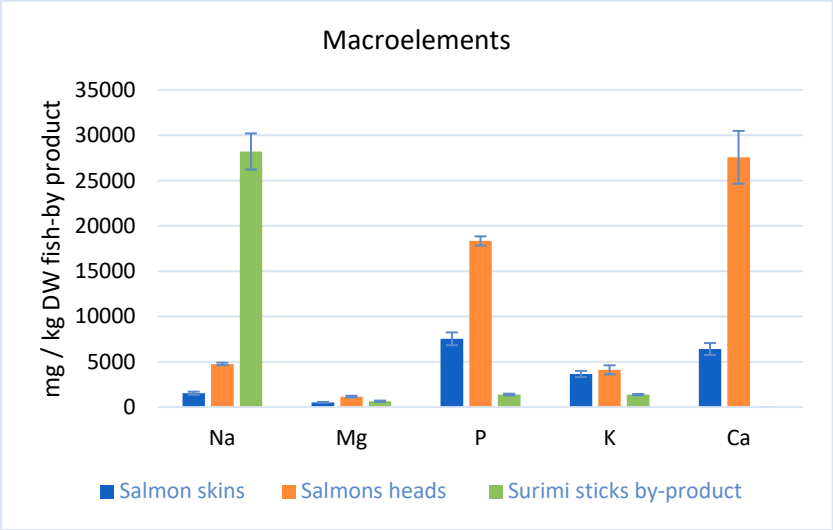

Figure 12. Macroelements content of salmon skins, salmon heads and surimi sticks by-product.

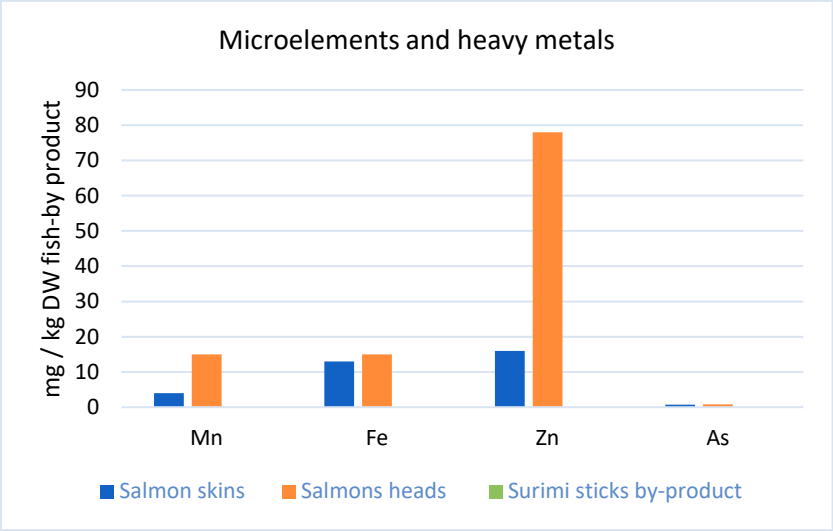

Figure 13. Microelements and heavy metals content of salmon skins, salmon heads and surimi sticks by-product. Microelements and heavy metals under the detection limits (Cr, Cu, Se, Mo, I, Cd, Hg, Pb) are not represented.

Salmon skins and heads were quite high in calcium and phosphorus. For the heads 27564 mg/kg DW Ca, 18334 mg/kg DW P were determined, which is consistent with data from the bibliography. Non-compliant surimi sticks were rich in sodium (28211 mg/kg DW Na), because of the salt added for the manufacture of surimi. Heavy metals were not present in any of the by-products except for arsenic, which was detected in salmon skins and heads. The obtained values are low: 0.7 and 0.8 mg/kg DW, respectively, and below FAO/WHO maximum permissible limits.

Below, Table 11 shows the results of microorganisms determination, on FW basis.

Table 11. Microbiology of fish by-products

Microorganism	Units on FW	Salmon skins	Salmon heads	Surimi sticks by-product
E. coli (*)	*E. coli* in 15g (CFU/g)	< 40	< 40	< 40
Salmonella	*Samonella* spp in 15g	ND	ND	ND
Total aerobic count	Total Aerobic count (CFU/g)	1.43×10^5	3.60×10^5	2.20×10^3
Total fungi and yeast count	Total Fungi and Yeast count (CFU/g)	1.74×10^3	1.04×10^4	< 40

(*) This method does not detect E. coli O157, as most of them are atypical and are not glucuronidase negative, and therefore do not produce a blue precipitate that facilitates counting in 3M Petrifilm EC plates.
ND: Not detected

Samples were below the permit limit (R.D.135/2010 B.O.E 25/02/2010) for frozen fishery products) for total aerobic count, and also for yeast and mould (< 10^{6} ufc/g) and *Salmonella* (absence in 25 g).

3.3.1 Fish-by products selection

The three fish by-products were selected to be further processed in Task 3.4 to produce functional Fish Protein Hydrolysates (FPHs). The main reason was that all the three biomasses were rich in protein, and they should be studied in further detail to determine the protein extraction yield. Thus, the biomass for the production of the preliminary ingredients for WP4 will be selected based on the protein extraction yield in Task 3.4, taking into account additional considerations such as the practicality of collection and the need for valorisation.

4. Conclusions

For each INNOAQUA biomass, a proximate composition (moisture, ash, lipid, protein contents) was performed. The description of the different biomasses was provided with special focus to the nutritional composition. The most promising biomass were selected as described below. The characterisation detailed in the present document, together with the expected extraction yields were used to estimate how much ingredients and biomass will be necessary for the development and production of the food products in WP4.

4.1 Microalgae

An exhaustive characterisation of biomass of the six initial strains has been performed, including total protein, lipids, carotenoids and carbohydrates content and the determination of the profile of fatty acids and carotenoids. The B-vitamin content of the selected strains has also been determined for the selected strains. The results, combined with the culture growth rates, were used to select one strain for each specie, which are the ones that are being produced in larger amounts for the INNOAQUA project development. The Norwegian **local *P.***

***tricornutum* B58 and the *C. sorokiniana* UTEX 1230 were selected due to their considerably higher productivity of protein 31-43% DW, PUFA's 6-8% and carotenoids 0.5-1% DW**. For the *N. oculata* strains, the difference among them were narrow, but **NIVA-3/04 was selected as the productivity indexes were slightly higher for protein and PUFA's**.

4.2 Macroalgae

Significant effort of strain selection and characterisation has been performed, mainly with *Ulva*, but also with *Gracilaria*. The protein results for ***Ulva*** (≈12% DW) are still considered lower than what was expected. For ***Gracilaria*** (protein 8.2% DW), the agar content will still be validated but it is estimated at approximately 30-35% minimum. Different strains of *Gracilaria* did not present important differences in chemical composition.

For these reasons, even though the up-scaling of biomass of the selected *Ulva* and *Gracilaria* strains is ongoing, more strains will continue to be screened for growth performance and nutrient utilization. If potentially interesting, some of those will then be characterised and can then be used in future tasks. In any case, that continued strain screening for *Ulva* and *Gracilaria gracilis* does not have any implications in planned tasks, such as task 3.3, extraction of ingredients and deliveries to the partners.

Finally, worth to stress the future work with *Porphyra*. The genus is highly valued in world aquaculture and there virtually no aquaculture production of this in Europe. *Porphyra* biomass can also have considerable amounts of protein, eventually higher than *Ulva*, when produced in the right conditions. That is the reason why it is included in INNOAQUA in the first place. In this case, however, the decision to use it or not as a source of protein will be strictly financial, *i.e.*, if the value of its protein compensates the extraction costs or if its use as whole ingredient/food is preferable.

4.3 Fish by-products

The characterisation revealed that all fish by-products were rich in protein (30% DW for salmon skins, 22% DW for salmon heads, 28% DW for surimi), which is crucial for producing Fish Protein Hydrolysates. The most abundant amino acids found in salmon skins and heads were glutamic acid, glycine, aspartic acid and arginine, while in surimi the most abundant amino acids were glutamic acid, aspartic acid, leucine and proline. Surimi showed high content of carbohydrates (66% DW). Salmon skins and heads were quite high in calcium and phosphorus, mainly in heads with 27564 mg/kg DW, 18334 mg/kg DW, respectively, while non-compliant surimi sticks were rich in sodium, 28211 mg/kg DW. Cadmium, mercury, and lead were not present in fish by-products, but low values of arsenic (under the permit limits) were found in salmon skins and head. *E. coli* and *Salmonella* were not detected in fish-by products.

The three fish by-products were selected to be further processed in Task 3.4 to produce functional Fish Protein Hydrolysates (FPHs) because all the three biomasses were promising in terms of protein content (22%-30% DW). They will be selected based on the protein extraction yield in Task 3.4, while taking into account additional considerations such as the practicality of the collection and the need for valorisation.

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