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D1.1. Feedstocks of the VALORISH process

VALORISH

GREEN VALORISATION CASCADE APPROACH OF FISH WASTE AND BY-PRODUCTS THROUGH FERMENTATION TOWARDS A ZERO-WASTE FUTURE

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EXECUTIVE SUMMARY

The present report exposes a chemical evaluation of a variety of fish by-products identified and generated in different fish industry factories: PESCANOVA Spanish factories and some ANFACO-CECOPESCA affiliated Spanish companies.

The chemical composition analysed included the determination of pH, moisture, ashes, total lipids, total proteins, total carbohydrates, total sugars and salt content. For by-products with a significant fat composition, fatty acid profile is also given.
When possible, different batches of the same raw material were analysed and, in such cases, experimental data is shown as average data, in order to identify potential variations in nutritional composition. These variations may appear due to non-constant factors such as biological or seasonal changes of each specie, and differences between suppliers specifications.
It is important for the VALORISH project in general to understand that variations in composition of by-products will occur, as we are not working with selected and concrete materials, but rather with the real waste from real industries, that adapt their productions to the stock availability in each of their sectors.

Generated volumes for each by-product were also considered in order to establish a preliminary selection. It has been challenging to obtain exact figures for this point, since in some cases companies do not have accurate information waste by waste separately.

Finally, by taking into consideration both nutritional profile and generated amount, a feedstock preselection was made for both oil extraction and proteolytic fermentation.

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LIST OF ACRONYMS

Abbreviation	Definition
ABP	Animal by-product
CF	Conversion Factor
CH	Carbohydrates
Coll	Collagen
EU	European Union
FAs	Fatty acids
FAME	Fatty Acid Methyl Esters
GC-FID	Gas Chromatography with Flame Ionization Detector
ICP-OES	Inductively Coupled Plasma Optical Emission Spectroscopy
Lip	Lipids
Moi	Moisture
n.d.	No data
NMR	Nuclear Magnetic Resonance
RF	Radio Frequency
Prot	Proteins
Sug	Sugar

1. INTRODUCTION

1.1 GENERAL WORLDWIDE CONTEXT

Food industry in general, and seafood industry in particular, generates food loss and waste at every stage of the value chain (Figure 1). As a result, there is a wide range of by-products of varying types, composition and quantities, depending on processes, volumes and formats of each factory that are traditionally used in other industries when possible (fishmeal and fish oil mainly).

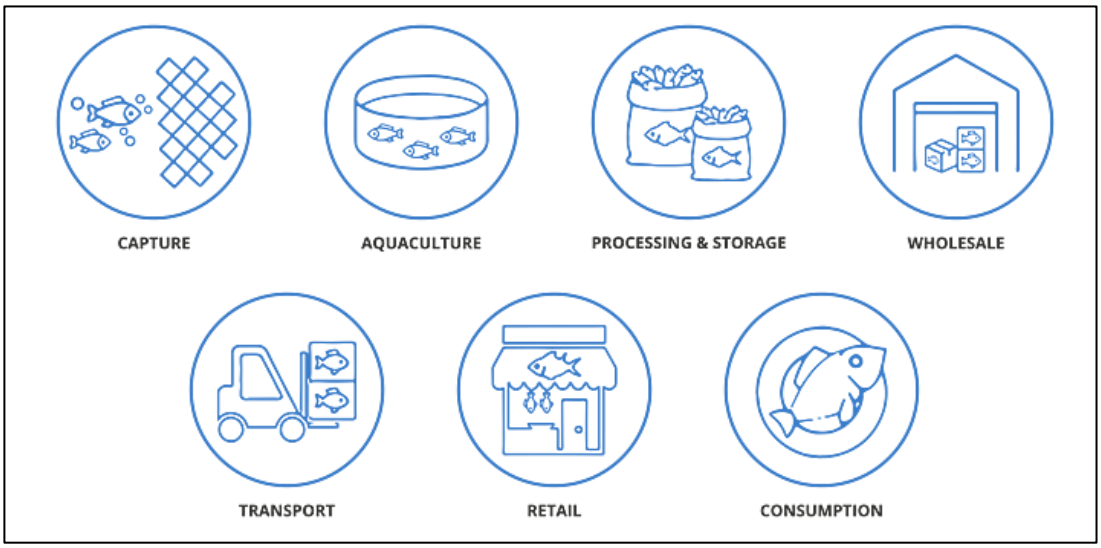

Figure 1. FAO infographic exploring Food Loss and Waste in the whole Fish Value Chain. (1)

The variety of operations that are executed specifically in the processing stage, generates a lot of by-products such as heads, tails, fins, skins, viscera, trimmings, little portions, etc. According to IFFO (2) , these wastes can account for 30 to 70% of the fish wet weight (Figure 2). That proportion, imply a huge amount of economic loss.

All the discards are potentially susceptible to be reused and valorised. And so they are to a certain degree. As previously pointed out, most of them are intended for fishmeal and fish oil, but they can also be intended for petfood, pharmaceutical products, nutraceuticals, fertilizers, agrochemicals or even energy production as well.

The VALORISH project focuses its main goal on the processing fishery industry, and aims to revalue its different by-products by obtaining bioproducts that may be returned and reincorporated into the food industry for human consumption in form of additives, supplements or nutraceuticals.

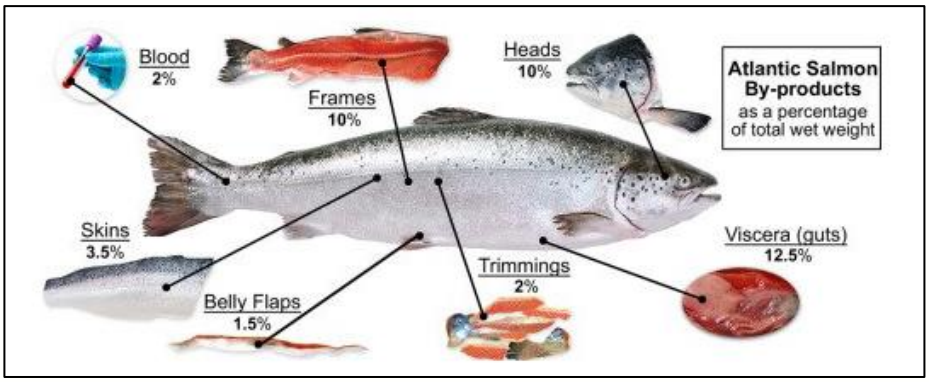

Figure 2. IFFO infographic showing different by-products from a whole fish. (2)

In this report, we include three different types of fishery products that are produced in that mentioned particular step (processing), depending on their distribution to the consumer:

- Processed chilled products: such as cooked shrimp or light salted cod, these products are known for being perceived as fresher products.
- Processed frozen products: such as breaded hake products or surimi products, they maintain the same nutritional properties as refrigerated version but offer a longer shelf life and they are usually cheaper than refrigerated ones.
- Canned products: such as canned tuna or sardines. They represent a significant part of the market, as they have the longest shelf life and they are usually ready to eat products, making them very convenient to use for the consumer.

According to a research from ANFACO-CECOPESCA in 2023 (3) the total production of processed fish products in Spain was 1.001.797 tons, where prepared and canned represent approximately 41%, frozen fish is near 19%, molluscs in different presentations are around 20%, crustaceans with diverse preparations suppose over 6%, chilled fish means around 4,6% and brined, salty or smoky fish constitute near 3% of the total. The remaining fraction consists basically of fishmeal or mass unfit for human consumption.

1.2 LEGAL FRAMEWORK

According to European regulation (4) the term "**Animal By-Products (ABP)"** refers to entire bodies or parts of animals, products of animal origin or other products obtained from animals, which are not intended for human consumption, including oocytes, embryos and semen.
ABP are classified into specific categories which reflect the level of risk to public and animal health. It is not permitted to use any animal by-product for human consumption, but Category 3 (low risk) ABP are authorised for producing fishmeal, fish oil, pet food or animal nutrition.

The raw materials selected for the VALORISH project are feedstocks with high potential for upcycling and valorising, but they are currently classified as Category 3 ABP. This means that for finally upcycle and revalorise them in the food industry for human consumption as supplements, nutraceuticals or additives, a change in their categorisation will be firstly needed in their generation step, defining them as food-grade side streams or even co-products instead of being declared "Category 3 ABP".

To this end, it is crucial that all the selected feedstocks meet food-grade quality standards. According to this, they should be maintained under the same hygienic and sanitary conditions as those designated for human consumption, in line with EU regulations.

VALORISH is aware that it would be necessary to verify the legal framework in each country where this research may have real application.

1.3 WPS AND TASKS RELATED WITH THE DELIVERABLE

This deliverable refers to "Task 1.1 Feedstock analysis, selection and preparation for processing" leaded by PESCANOVA. In turn, this first Task is included in "WP1: Feedstock management, extraction and hydrolysis biorefinery technologies", whose leading and managing partner is ANFACO-CECOPESCA.

2. MATERIAL AND METHODS

2.1 Raw materials and available by-products

The project has managed to have several by-products available for evaluation from different Spanish companies off the fishing industry.
Most common operations from those factories, include de-heading, de-boning, skin removal, gutting, de-finning, cutting, chopping, cooking, brining, salting, canning or packaging.
Some generated by-products in those working environments, are nowadays directly destructed by an authorized waste manager. Others are currently derived and re-used in other industries such as fishmeal, fish oil, animal feed, petfood or chemical industry (Table 1).

Nevertheless, the aim of VALORISH is to go further and do valorisation of those industrial by-products and re-introduce them in the food chain.
Either PESCANOVA and ANFACO-CECOPESCA affiliated factories, provided the necessary raw materials to carry out a review and report of their available by-products that might potentially be used as Feedstocks in the VALORISH project.

*Table 1. By-products types, their actual destination and generated volumes in PESCANOVA own factories, ANFACO-CECOPESCA associated factories and NOFIMA's collaborators during 2024. * Salmon bones and cod backbones data are from the entire country of Norway in 2022 (5).*

Partner	Supplying factory	By-product	Actual destination	Annual tons
PESCANOVA factories	Catarroja factory	Shrimp	Fishmeal	97
		Cephalopods	Destruction	33
	Paterna factory	Cod	Fishmeal	45
	Chapela factory	Surimi	Fats and proteins for animal nutrition, aquaculture, petfood, fertilizers and chemical industry	764
	Porriño factory	Hake sawdust	Biogas valorisation	≈ 45
ANFACO affiliated factories	Canning factory	Sardine	Fishmeal	70
	Canning factory	Tuna dark muscle/meat	Fishmeal / Petfood	n.d.
	Fish processing factory	Trout	n.d.	n.d.
	Fish processing factory	Blue-shark	Animal feed	20
	Smoked fish processing factory	Salmon	Fishmeal	1600
NOFIMA's collaborators	Fish processing factory	Salmon bones	Bonemeal for animal feed.	7500*
	Marine ingredientes processor	Cod backbones	Fishmeal	10000*

2.2 Chemical analysis

Potential by-products were identified both from PESCANOVA factories, ANFACO-CECOPESCA associated companies and NOFIMA's collaborators for their characterisation since they fit the project objectives (Figure 3).

The main objective of this characterisation was to know the nutritional composition of the residues to make a selection and assign them in subsequent tasks and work packages of the project, either lipidic extraction or proteolytic fermentation.

Nutritional analyses were performed by an external laboratory in case of PESCANOVA company, whereas ANFACO and NOFIMA carried out tests by themselves.

- **Moisture (gravimetric method)**: In-house method. One of the most common methods of water content determination is gravimetric method with oven drying. This method involves weighing a moist sample, oven drying it at 105°C for 24-48 h, reweighing, and calculating the mass of water lost as a percentage of the mass of the dried sample.
- **Protein (Kjeldahl method)**: In-house method. The Kjeldahl method is a laboratory procedure for determining the amount of nitrogen and protein in a sample. It involves digesting the sample in sulfuric acid, distilling the ammonia, and then titrating the ammonia with a standard acid (HCl 0,1N). The official method uses the coefficient 6,25 for most natural products. This ratio comes from the assumption that proteins of different raw materials contain around 16% of nitrogen. So, the value of total nitrogen (%) obtained multiplied by the coefficient 100/16 (6,25), leads us to percentage of crude protein:

  % Crude Protein (Dry basis) = 6,25 x % Nitrogen (Dry basis).

  % Crude Protein (Wet basis) = 6,25 x % Nitrogen (Wet basis).
- **Protein (Dumas method):** In-house method based on UNE-EN ISO 16634. The method consists of combusting a sample of known mass to a temperature between 800 and 900 °C in the presence of oxygen. This leads to the release of carbon dioxide, water and nitrogen. The gases are then passed over special columns (such as potassium hydroxide aqueous solution) that absorb the carbon dioxide and water. A column containing a thermal conductivity detector at the end is then used to separate the nitrogen from any residual carbon dioxide and water and the remaining nitrogen content is measured. The measured signal from the thermal conductivity detector for the unknown sample can then be converted into a nitrogen content. A 6,25 coefficient is also used, as in Kjeldahl method.
- **Ash (gravimetric method)**: It involves the complete incineration of a food sample to remove all organic matter, leaving behind only the inorganic residue or ash. The weight of this residue is then measured and expressed as a percentage of the original sample weight.
- **Fat Nuclear Magnetic Resonance (NMR)**: In-house method based on AOAC 2008.06. The method includes a microwave-drying step followed by NMR analysis. Moisture is evaporated from the sample by using microwave energy. Weight loss is determined by electronic balance readings before and after drying and is converted to moisture content by the system's microprocessor. Fat is determined by pulsed Radio Frequency (RF) energy while within a static

magnetic field. The resulting NMR signal is recorded and analysed for the total proton activity of fat present in the sample.

- **Fat (Soxhlet)**: In-house method. Fat is extracted from the sample using ethyl ether. Then, the solvent is evaporated and the fat is determined gravimetrically.
- **Relative composition of fatty acid by Gas Chromatography with Flame Ionization Detector (CG-FID)**: In-house method. Phospholipids are hydrolysed to free their fatty acids from the sn-1 and sn-2 glycerol backbone. These fatty acids undergo methylation of the carboxylic acid function to form Fatty Acid Methyl Esters (FAME). This hydrolysis and methylation occur at room temperature when reacted with 1N sodium methoxide. The FAMEs are identified and quantitated using capillary GC-FID.
- **Salt content (from sodium)**: Method according to Regulation (EU) nº 1169/2011. Calculation from sodium, considering a 2,5 factor to sodium chloride.
- **Sodium by atomic absorption spectroscopy (flame atomization)**: In-house method based on UNE-EN 13804. The sodium is determined after mineralisation by dry process by atomic absorption spectrometry. The addition of a spectral buffer to avoid ionisation of sodium is necessary.
- **Carbohydrates (by calculation)**: Method according to Regulation (EU) nº 1169/2011. Total carbohydrate values include total dietary fiber. This value was calculated by subtracting the sum of the percentages of water, protein, total lipid (fat), ash, total dietary fiber from 100.
- **Energy value (by calculation)**: Method according to Regulation (EU) nº 1169/2011. It uses a single factor for each of the energy-yielding substrates (protein, fat, carbohydrate), regardless of the food in which it is found. The energy values are 17 kJ/g (4,0 kcal/g) for protein, 37 kJ/g (9,0 kcal/g) for fat and 17 kJ/g (4,0 kcal/g) for carbohydrates.
- **Collagen**: ion chromatography based on ISO 13903:2005. Based on hydroxyproline, collagen was estimated by a conversion factor of 14, based on shark skins.
- **Amino acid**: Amino acid composition of the fish bones was determined according to AOAC Official Method 2018.06 (6) with some adjustments. The samples were hydrolyzed in 6 M HCl for 22h at 110°C. L-Norvaline (Sigma-Aldrich) was added as internal standard. The UPLC analysis was conducted on a Waters ACQUITY UPLC (ultra performance liquid chromatography) I-Class PLUS with fluorescence detector (Waters, Milford, MA, USA). The separation of the amino acids was done on a Waters AccQ-Tag Ultra column (2,1 x 100 mm, with a particle size of 1,7 µm) (Waters, Milford, MA, USA). For derivatization of amino acids, 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) supplied by Waters (Waters AccQ-Tag Ultra derivatization kit, Waters, Milford, MA, USA) was used. Waters AccQ-Tag Ultra eluent A and B (Waters, Milford, MA, USA) was used as mobile phases. The flow rate was 0.7 mL/min and the solvent gradient was set as follows with curve 6 unless mentioned otherwise: 0,0-0,54 min 99,9% A and 0,1% B; 0,54-5,74 min 90,9 % A and 9,1 % B (curve 7); 5,74-7,74 min 78,8% A and 21,2% B; 7,74-8,04 min 40,4% A and 59,6 % B; 8,04-8,05 10% A and 90% B; 8,05-8,64 10% A and 90% B; 8,64-9,00 99,9% A and 0,1% B; 9,00-10,00 99,9% A and 0,1% B. The column temperature was set to 43°C to ensure optimal separation of all amino acids including Hydroxyproline. The detection of the amino acids was carried out using a fluorescence detector

with wavelengths for excitation and emission set at 266 and 473 nm, respectively. The total analysis time was 10 minutes.

- **Minerals**: Minerals (Ca, Cu, K, Mg, Mn, Na, P, and Zn) in the fish bones were analysed using Agilent 5110 VDV (Vertical Dual View) ICP-OES (Inductively Coupled Plasma Optical Emission Spectroscopy) (7) and according to Norwegian Standard NS-EN 15621:2017 with some adjustments. The homogenized sample was added ultrapure water (resistivity 18,2 MΩ·cm at 25°C) (1 mL) and nitric acid (HNO_3) (69%, 2 mL), and digested in a Milestone UltraWave at 220°C and 110 bar for 15 minutes after a 20-minute warm-up time. The extracts were diluted with ultrapure water, and the element concentration in the resultant solution was determined using ICP-OES attached to an autosampler (Agilent SPS4) by using external calibration. Selenium (Se) was determined according to the same principle, but with a prereduction step before ICP-OES analysis to reduce SeVI to SeIV according to Norwegian Standard NS-EN 16159:2012 with some adjustments. The prereduction was carried out by adding hydrochloric acid (HCl, 32%, 5 mL) to an aliquot of the digested and diluted sample (10 mL), and heating in water bath at 100°C for 10 minutes. After final dilution, the selenium was analysed on the ICP-OES by using MSIS (Multimode Sample Introduction System) according to Drvodelic 2017, where SeIV in the sample reacts with a reductant solution (1,5 % $NaBH_4$ in 0,1 % NaOH) to form hydrogen selenide (SeH_2).

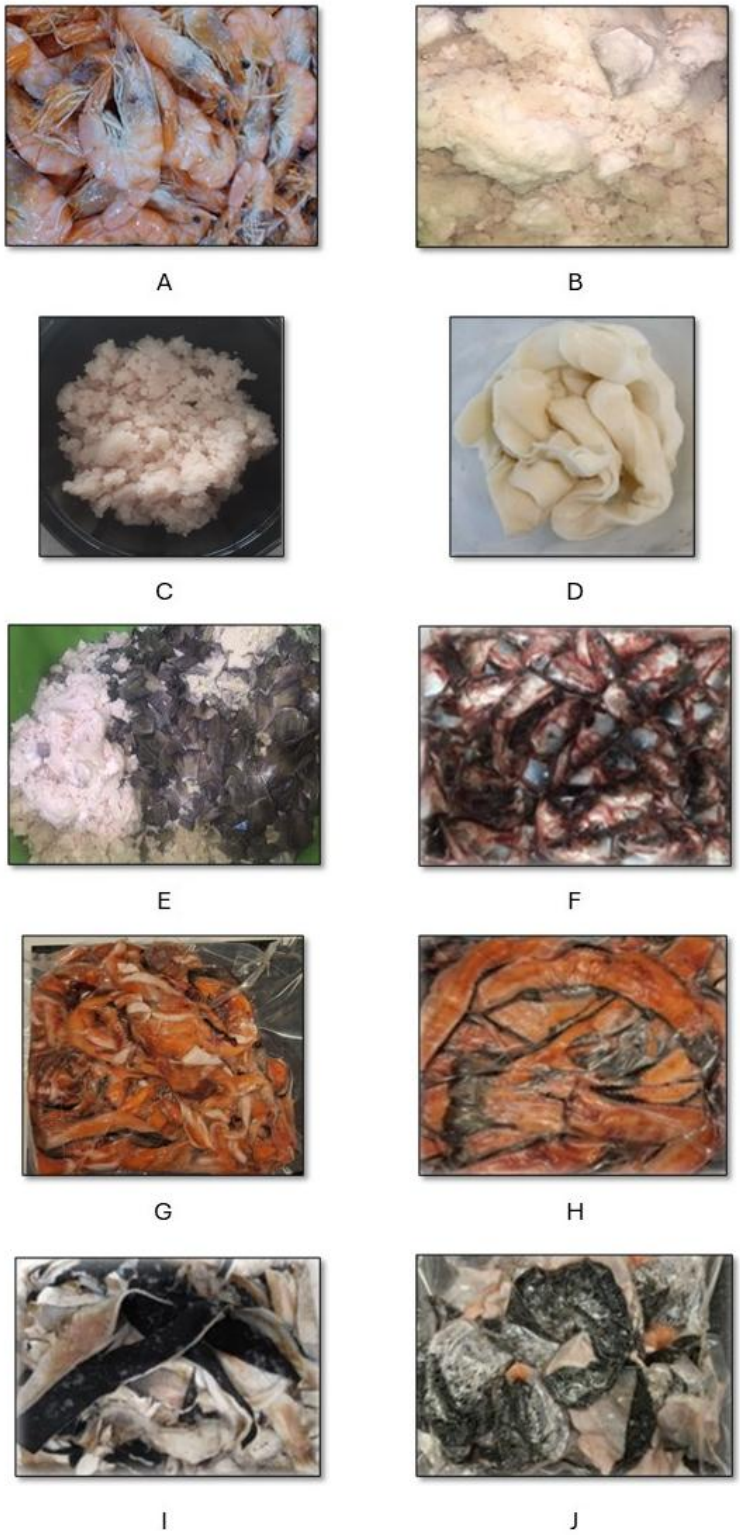

Figure 3 Available by-products. A: Shrimp. B: Cephalopods. C: Hake sawdust. D: Surimi dough remains. E: Cod. F: Sardine. G: Tuna dark muscle. H: Trout. I: Blue shark. J: Salmon.

3. RESULTS AND DISCUSSION

3.1 Shrimps

The shrimp *Litopenaeus vannamei* cooked in a brine generates a by-product composed of whole non-compliant pieces with dark heads for instance, that suppose almost 100 T of industrial waste annualy in the Catarroja factory, from PESCANOVA Group.

Since this waste must be considered as all in one: shrimp meat, head, and entire shell, ash figure shown in Table 2 is one of the highest of the available samples: (21,15% in dry basis).

Increased values of salt content derived from the cooking process (in brine), might contribute to ash figures as well.

The shrimp meat constitutes 54% of the whole shrimp whereas the shrimp shell represents 46% of it according to PESCANOVA internal data. There is no capacity in this particular factory to process both residues separately, but it might be a viable option for other companies where this research may apply.

Whereas fats are not significant in this by-product, protein content is still important even considering the mentioned ratio between shrimp meat and shrimp shell.
The nitrogen-protein conversion factor used in all analyses was the traditional 6,25 used in Kjeldahl Method. The VALORISH team is aware of the potential error in this number specifically to shrimp by-products where, as mentioned above, the available by-product is the whole shrimp (including shell), as Nx6,25 is generally used for crude protein content only. We do not have the amino acid composition of the sample, and it is not the objective of this Task, so even though we are aware of the potential error that this number may entail, we accept 6,25 as CF.

Table 2. Nutritional composition for Shrimp by-products. n=3.

pH		7,2
Moi (%)	Wet basis	77,43 ± 1,06
Ash (%)	Wet basis	4,73 ± 1,46
	Dry basis	21,15 ± 7,35
Lip (%)	Wet basis	<0,50 ± 0,00
	Dry basis	<2,22 ± 0,10
Prot (%)	Wet basis	13,83 ± 4,21
	Dry basis	61,01 ± 16,57
CH (%)	Wet basis	3,30 ± 4,85
	Dry basis	14,72 ± 21,66
Sug (%)	Wet basis	<0,50 ± 0,00
	Dry basis	<2,22 ± 0,10
Salt (%)	Wet basis	1,83 ± 0,18
	Dry basis	8,10 ± 0.54

According to nutritional profile observed, shrimp by-product might be a good candidate for proteolytic fermentation.

3.2 Cephalopods.

After the chopping process for obtaining cut or sliced cephalopods products this by-product is generated, consisting mainly of mass remains and sawdust from the cutting machinery.

This by-product has a very high moisture content, as well as protein values (70,01% in dry basis), as observed in Table 3.

Table 3. Nutritional composition for Cephalopods by-products. n=2.

pH		8,8
Moi (%)	Wet basis	84,60 ± 8,77
Ash (%)	Wet basis	1,35 ± 0,49
	Dry basis	11,55 ± 9,79
Lip (%)	Wet basis	1,25 ± 1,06
	Dry basis	7,35 ± 2,70
Prot (%)	Wet basis	11,55 ± 8,84
	Dry basis	70,01 ± 17,54
CH (%)	Wet basis	<0,50 ± 0,00
	Dry basis	<3,87 ± 2,21
Sug (%)	Wet basis	<0,50 ± 0,00
	Dry basis	<3,87 ± 2,21
Salt (%)	Wet basis	0,28 ± 0,20
	Dry basis	1,73 ± 0,29

As a consequence of these high protein values and low-fat results, this waste could be a good option for proteolytic fermentation.

The manufactured volume of cephalopods products in this factory is quite low (as observed in Table 1), and condensed only in certain seasons of the year, so it is not always available.

3.3 Cod.

From the manufacturing process of fish loins, fillets and portions, cod by-products (consisting of little crumbles, portions and some tails) are generated.
It is important to note that they may be salted or light salted.

Analytic results show the highest content in salt (17,28% in dry basis), and even the highest ash content, probably derived from the salt amount.
As shown in Table 4, there is a wide variation in both values, salt and ashes, that may be due to the possible coexistence in the factory of “salted cod” or “light salted cod” productions. All generated cod discards were deposited together in a waste container and collected collectively without being separated, according to the standard waste management procedure in Paterna factory.

Table 4. Nutritional composition for cod by-products (little crumbles, portions and tails). n=3.

pH		6,3
Moi (%)	Wet basis	78,37 ± 6,24
Ash (%)	Wet basis	5,87 ± 7,65
	Dry basis	22,62 ± 25,13
Lip (%)	Wet basis	1,13 ± 1,10
	Dry basis	5,59 ± 5,54
Prot (%)	Wet basis	14,13 ± 2,77
	Dry basis	70,45 ± 27,13
CH (%)	Wet basis	<0,50 ± 0,00
	Dry basis	<2,44 ± 0,66
Sug (%)	Wet basis	<0,50 ± 0,00
	Dry basis	<2,44 ± 0,66
Salt (%)	Wet basis	4,67 ± 7,03
	Dry basis	17,28 ± 23,92

Since fat values for this by-product are quite scarce and content in proteins is high, it may be a viable alternative for proteolytic fermentation.

It is important to take those mentioned wide variations regarding salt content into consideration for fermentative bacteria selection and bacterial growth.
If necessary, salted cod and light salted cod could be separated directly in the plant. This will undoubtedly imply additional costs due to factory scheduling, and it would be interesting to evaluate the efficiency of the process.

3.4 Surimi.

It is important to highlight in this case that this by-product consists of a residue of an already composed product, not a little processed material such as salty fish or cooked shrimp.
Frozen surimi blocks in the factory are mixed with the rest of ingredients such as starch, oil, salt, flavourings, etc., in order to manufacture a Surimi-based product, an analogue product usually, including surimi crabsticks for instance.
It is from that surimi-based product, from where the by-product is generated.

The impact of this complex matrix with the ingredients mix could be appreciated in the analytical data presented in Table 5. As a result, proteins are slightly lower than expected when compared to the surimi blocks without any other component. Actually, the obtained data show the lowest number of proteins of all assessed by-products (29,38% in dry basis).

Fats are also one of the lowest values (3,14% in dry basis), wich make the surimi waste not a good candidate for oil extraction.

On the opposite side, carbohydrates, represent a great part of the composition of this by-product (57,25% in dry basis) including sugar (over 10% in dry basis). CH levels in surimi by-products are the highest of all our available feedstocks by far, mainly derived from starch used in the surimi product.
This, added to the high salt values (7,8% in dry basis), may be relevant for bacteria selection and bacterial growth in proteolytic fermentation.

Table 5. Nutritional composition for surimi by-product. n=3.

pH		6,7
Moi (%)	Wet basis	77,07 ± 0,12
Ash (%)	Wet basis	1,97 ± 0,05
	Dry basis	8,59 ± 0,25
Lip (%)	Wet basis	0,72 ± 0,17
	Dry basis	3,14 ± 0,76
Prot (%)	Wet basis	6,73 ± 2,29
	Dry basis	29,38 ± 10,04
CH (%)	Wet basis	13,13 ± 2,66
	Dry basis	57,25 ± 11,44
Sug (%)	Wet basis	2,43 ± 1,38
	Dry basis	10,60 ± 5,98
Salt (%)	Wet basis	1,80 ± 0,15
	Dry basis	7,83 ± 0,67

Surimi by-product might also present a little amount of fiber content in its composition (around 1% in wet basis), most likely coming from the use of vegetal starches during the processing, even when no added fiber as an ingredient itself in the formula. It is related with the “resistant starch” concept, where some starches resist degradation during the heat treatment (8). As there is no real fiber, we did not include it in the chemical characterisation.

3.5 Hake.

Consisting of sawdust and little crumbles from cutting hake fishblocks, for producing hake breaded products in Porriño factory.

Presented data in Table 6, manifest one of the highest salt contents, which also seems to affect to ashes value.

It is important to note that salt content in this by-product may vary depending on the raw material used in the factory: Whereas some of the minced suppliers process the fishblocks with fresh water, there are some others that use sea water to carry out that process in their facilities, and that is the reason why deviations in salt content are so remarkable.

To this concrete case it is not possible to separate both residues (salty raw material and less salty raw material) in the factory, as the processing plant may be producing at the same time in various lines with different raw materials.

Carbohydrates are not relevant in this case, as this by-product comes from just white fishblock waste. Likewise, fats are not weighty. Regarding proteins, values around 70% in dry basis were measured.

Table 6. Nutritional composition for hake sawdust by-product. n=3.

pH		6,65
Moi (%)	Wet basis	81,23 ± 0,90
Ash (%)	Wet basis	2,70 ± 2,17
	Dry basis	14,72 ± 12,41
Lip (%)	Wet basis	1,20 ± 0,61
	Dry basis	6,31 ± 3,05
Prot (%)	Wet basis	13,23 ± 1,59
	Dry basis	70,39 ± 6,04
CH (%)	Wet basis	<0,50 ± 0,00
	Dry basis	<2,67 ± 0,13
Sug (%)	Wet basis	<0,50 ± 0,00
	Dry basis	<2,67 ± 0,13
Salt (%)	Wet basis	2,12 ± 2,71
	Dry basis	11,67 ± 15,28

Having reviewed nutritional profile, the suggestion for hake sawdust would be to use it in proteolytic fermentation, taking into consideration variation in salt content values for bacteria selection.

3.6 Sardine.

Made up of discards that are originated in the sardine canning industry. These by-products consist mainly of fresh heads and tails, from the processing of the fish before the boiling process.

As observed in Table 7, sardine by-products have an elevated protein content (near 58% in dry basis).

Carbohydrate content and salt content are relatively low. Ashes are present in a considerable amount, most likely due to the contribution of bones from sardine heads.

Table 7. Nutritional composition off sardine by-product. n=1.

pH		6,60
Moi (%)	Wet basis	76,00
Ash (%)	Wet basis	5,30
	Dry basis	22,08
Lip (%)	Wet basis	3,60
	Dry basis	15,00
Prot (%)	Wet basis	13,90
	Dry basis	57,92
CH (%)	Wet basis	1,20
	Dry basis	3,13
Salt (%)	Wet basis	0,45
	Dry basis	1,88

In this case, fat content is higher than in previous characterized by-products, even though it is still considered a moderate amount of fat, and higher fatty by-products are intended. All feedstocks with medium to high oil content will be considered for oil extraction purposes and, for that reason, fatty acid composition is also described, with the results shown in Table 8 for sardine by-products.

Table 8. FAs composition for sardine by-product. n=1.

Saturated FAs (%)	1,10
Monounsaturated FAs (%)	0,90
Polyunsaturated FAs (%)	1,00
EPA + DHA (%)	0,77
Omega 3 (%)	0,90
Omega 6 (%)	0,06
Trans (%)	0,12
Fat acidity (%)	6,00

In view of the obtained results, sardine by-products seem to be a possible option for both oil extraction or proteolytic fermentation.

3.7 Tuna dark muscle.

Tuna cannery industry is the most important considering the entire cannery sector, representing nearly 73% of the total volume of canned products in Spain (3).
Apart from the typical waste generated in all canneries (heads, bones or viscera), tuna canning also generates the well-known tuna dark muscle or tuna dark meat.

Table 9 manifest that tuna dark muscle is a discard with high protein values, as well as noticeable fats and carbohydrates.

Table 9. Nutritional composition for tuna dark muscle by-product. n=1.

pH		5,60
Moi (%)	Wet basis	69,00
Ash (%)	Wet basis	0,20
	Dry basis	0,65
Lip (%)	Wet basis	4,10
	Dry basis	13,23
Prot (%)	Wet basis	21,20
	Dry basis	68,39
CH (%)	Wet basis	4,40
	Dry basis	13,65
Salt (%)	Wet basis	1,27
	Dry basis	4,10

As previously mentioned, feedstocks with high oil content will be likely intended for the oil extraction task, so for the samples with highest lipids content (>10% in dry basis), fatty acid composition is also described, with the results shown in Table 10 for tuna by-product:

Table 10. FAs composition for tuna dark muscle by-product. n=1.

Saturated FAs (%)	1,60
Monounsaturated FAs (%)	1,20
Polyunsaturated FAs (%)	0,80
EPA + DHA (%)	0,58
Omega 3 (%)	0,66
Omega 6 (%)	0,13
Trans (%)	0,07
Fat acidity (%)	17,60

According to observed data, it might be a good alternative either for oil extraction or proteolytic fermentation.

3.8 Trout.

Consisting of fresh heads, bones and trimmings coming from trout filet processing.

No matter the sort of by-product, Table 11 shows that trout has very high fat values (44,38%, 41,18%, 48,18%) and relatively noticeable protein values (43,44%, 45,00%, 45,76%) in dry basis. Low carbohydrate content and low salt content, added to mentioned fat and protein values, make this by-product very interesting for valorisation either in oil extraction or proteolytic fermentation.

Table 11. Nutritional composition for trout different by-products. n=1.

		Heads	Bones	Trimmings
pH		6,80	6,80	6,60
Moi (%)	Wet basis	68,00	66,00	67,00
Ash (%)	Wet basis	3,80	4,60	1,57
	Dry basis	11,88	13,53	4,76
Lip (%)	Wet basis	14,20	14,00	15,90
	Dry basis	44,38	41,18	48,18
Prot (%)	Wet basis	13,90	15,30	15,10
	Dry basis	43,44	45,00	45,76
CH (%)	Wet basis	0,10	0,10	0,40
	Dry basis	0,31	0,29	1,21
Salt (%)	Wet basis	0,29	0,20	0,20
	Dry basis	0,91	0,59	0,61

Fatty acid composition is also presented in Table 12 for trout heads, bones and trimmings.

Table 12. FAs composition for trout different by-products. n=1.

	Heads	Bones	Trimmings
Saturated FAs (%)	2,20	2,20	2,50
Monounsaturated FAs (%)	6,90	6,60	7,40
Polyunsaturated FAs (%)	4,20	4,30	5,00
EPA + DHA (%)	0,90	0,93	0,94
Omega 3 (%)	1,60	1,63	1,70
Omega 6 (%)	2,50	2,55	3,16
Trans (%)	0,04	0,03	0,02
Fat acidity (%)	1,10	1,40	1,40

3.9. Blue shark.

From blue shark fishing and its subsequent skin removal processing for sale.

According to the results shown in Table 13, blue shark skin is a very protein-rich by-product (over 82% in dry basis).
Other components such as lipids or carbohydrates are not significant in this specific case.

What it stands out is the collagen value. Blue-shark by-product is the only one we have available with such a collagen content (46,5% in dry basis).

Table 13. Nutritional composition for blue shark skin by-product. n=1.

pH		7,30
Moi (%)	Wet basis	71,00
Ash (%)	Wet basis	4,80
	Dry basis	16,55
Lip (%)	Wet basis	0,13
	Dry basis	0,45
Prot (%)	Wet basis	24,00
	Dry basis	82,76
CH (%)	Wet basis	0,10
	Dry basis	0,34
Salt (%)	Wet basis	0,65
	Dry basis	2,24
Coll (%)	Wet basis	13,50
	Dry basis	46,54

This waste may be an excellent selection for protein recovery and collagen obtaining.

3.10. Salmon by-product

Consisting of heads and skin separately generated in salmon processing in one of the ANFACO-CECOPESCA associated factories.

Table 14 offers nutritional composition for salmon heads and salmon skin, showing high protein content and low carbohydrates and salt values.

This by-product (together with trout by-product seen in 3.8 section) is one of the by-products with the highest fat values (over 59% and 41% in dry basis in heads and skin respectively). Therefore, this may be a good candidate for oil extraction.

Table 14. Nutritional composition for salmon different by-products. n=1.

		Heads	Skin
pH		7,10	7,00
Moi (%)	Wet basis	59,00	56,00
Ash (%)	Wet basis	3,50	0,95
	Dry basis	8,54	2,16
Lip (%)	Wet basis	24,30	18,10
	Dry basis	59,27	41,14
Prot (%)	Wet basis	13,10	24,90
	Dry basis	31,95	56,59
CH (%)	Wet basis	0,10	0,10
	Dry basis	0,24	0,23
Salt (%)	Wet basis	0,56	0,52
	Dry basis	1,37	1,18

Fatty acid composition of salmon by-product is also presented in Table 15.

Table 15. FAs composition for salmon different by-products. n=1.

	Heads	Skin
Saturated FAs (%)	3,70	2,60
Monounsaturated FAs (%)	11,00	9,00
Polyunsaturated FAs (%)	7,40	5,40
EPA + DHA (%)	1,90	1,18
Omega 3 (%)	3,90	2,72
Omega 6 (%)	3,20	2,52
Trans (%)	0,09	0,06
Fat acidity (%)	1,30	1,60

3.11. Salmon bones and cod bones.

Although these two by-products will be assessed deeply in Work package 2, a brief overview of characterisation results made by NOFIMA is included.

The chemical composition of the bones revealed high levels of ash and protein (Table 16) on a dry matter basis, where the levels were similar between the two raw material types.

Table 16. Chemical composition of salmon head bones and cod backbone bones. n=1.

		Salmon bones	Cod bones
Moi (%)	Wet basis	49,9	53,0
Ash (%)	Wet basis	30,5	28,4
	Dry basis	60,9	60,4
Lip (%)	Wet basis	<1,5	<1,5
	Dry basis	<3,0	<3,0
Prot (%)	Wet basis	18,8	17,6
	Dry basis	37,5	37,4

The amino acid composition (Table 17) showed high levels of connective tissue-associated amino acids hydroxyproline, proline and glycine, indicating that the bones are rich in collagen proteins. The level of hydroxyproline was somewhat higher in salmon bones, suggesting that the levels of collagen may be higher in the salmon head bones compared to cod backbone bones.

Table 17. Amino acid composition of salmon head bones and cod backbone bones. n=1.

		Salmon bones	Cod bones
Hydroxyproline	Wet basis	1,3	0,81
	Dry basis	2,6	1,7
Alanine	Wet basis	1,4	1,4
	Dry basis	2,8	3,0
Arginine	Wet basis	1,2	1,3
	Dry basis	2,4	2,8
Aspartic acid	Wet basis	1,2	1,3
	Dry basis	2,4	2,8
Phenylalanine	Wet basis	0,4	0,38
	Dry basis	0,8	0,8
Glutamic acid	Wet basis	1,8	1,8
	Dry basis	3,6	3,8
Glycine	Wet basis	3,5	3,3
	Dry basis	7,0	7,0
Histidine	Wet basis	0,39	0,25
	Dry basis	0,8	0,5
Isoleucine	Wet basis	0,33	0,33
	Dry basis	0,7	0,7
Leucine	Wet basis	0,56	0,63
	Dry basis	1,1	1,3
Lysine	Wet basis	0,65	0,68
	Dry basis	1,3	1,4
Methionine	Wet basis	0,51	0,38
	Dry basis	1,0	0,8
Proline	Wet basis	1,7	1,6
	Dry basis	3,4	3,4

Serine	Wet basis	0,84	1,0
	Dry basis	1,7	2,1
Threonine	Wet basis	0,55	0,54
	Dry basis	1,1	1,1
Tyrosine	Wet basis	0,28	0,2
	Dry basis	0,6	0,4
Valine	Wet basis	0,49	0,49
	Dry basis	1,0	1,0
Cysteine/Cystine	Wet basis	<0,1	<0,1
	Dry basis	<0,2	<0,2
Tryptophan	Wet basis	<0,1	<0,1
	Dry basis	<0,2	<0,2
Sum amino acids	Wet basis	17,1	16,39
	Dry basis	34,1	34,9

The mineral composition (Table 18) showed that both types of bones were notably rich in calcium and phosphorus. These two minerals are components of the mineral structure hydroxyapatite ($Ca_5(PO_4)_3(OH)$), which gives the bones its rigid structure. Although cod had a higher total mineral content than salmon, the most notable difference between the samples was that salmon had six times and four times more zinc and manganese than cod, respectively.

Table 18. Mineral composition of salmon head bones and cod backbone bones. n=1.

		Salmon bones	Cod bones
Phosphorus (P) (mg/Kg)	Wet basis	62000	74000
	Dry basis	123752	157447
Potassium (K) (mg/Kg)	Wet basis	360	290
	Dry basis	719	617
Calcium (Ca) (mg/Kg)	Wet basis	120000	150000
	Dry basis	239521	319149
Cupper (Cu) (mg/Kg)	Wet basis	0,86	0,86
	Dry basis	2	2
Magnesium (Mg) (mg/Kg)	Wet basis	3200	3500
	Dry basis	6387	7447
Manganese (Mn) (mg/Kg)	Wet basis	46	11
	Dry basis	92	23
Sodium (Na) (mg/Kg)	Wet basis	1300	2400
	Dry basis	2595	5106
Zinc (Zn) (mg/Kg)	Wet basis	250	39
	Dry basis	499	83
Total minerals (mg/Kg)	Wet basis	187156,86	230240,86
	Dry basis	373567	489874

3.12. Turbot.

At the time the VALORISH Project proposal was presented, PESCANOVA was selling filleted turbot, so it was included as a possible by-product to be recovered and revalued. However, it is now a product that is sold completely whole, so there is no by-product availability. It was not possible to carry out the characterisation of this initial suggested by-product.

4. CONCLUSIONS

4.1 SELECTED BY-PRODUCTS FOR OIL EXTRACTION OR PROTEOLYTIC FERMENTATION.

Due to their highest oil content, the by-products with the highest potential for the **oil extraction**, would be sardine, salmon and trout by-products, especially the last two, which are the selected ones for that task, being heads the most available typology.

Based on protein results, shrimp, cephalopods, cod, hake, tuna and blue shark by-products would be the most interesting for **proteolytic fermentation**.
Protein recovery from whole shrimps, including shell and meat, might be attractive for industry waste management and sustainability purposes.
Nutritional profiles of cod (light salted cod), hake, and cephalopods are similar, as they consist basically of white fish “meat” or cephalopod “meat”.
Apart from being valued separately, it may be a good option to explore the possibility of revalorising them in a jointly way, going for the proteolytic fermentation with 2 or even all the 3 wastes together. Even the way they are generated (little portions, crumbles, trimmings and sawdust, without heads, viscera or shells) make this option feasible. By doing this, the volume of potential revalue would be over 120 T, aligning with VALORISH’s goal of recovering as much waste as possible, and helping to reduce industry residues.
Although dark muscle tuna was an interesting by-product, it is not as available as other by-products.
Since it is the only one with collagen content, it is suggested to reserve blue shark by-products for that purpose.

Even without having a high protein content, if we consider generated volumes, surimi (over 750 T/year) is a noticeable by-product to be revalued for protein recovery as well, from the industrial point of view (waste management) and from the perspective of having high availability.

Having all this comments into consideration, the final selection was:

- Feedstocks for oil extraction: Salmon heads by-product and trout heads by-product.
- Feedstocks for proteolytic fermentation: Shrimp by-products, surimi by-products, and hake or hake+cod or hake+cod+cephalopods (jointly) by-products.

With this final selection of feedstocks, the established KPI for this task of the project is met, identifying at least 5 different feedstocks with distinct composition from various industries to be upcycled and revalorised, representing the heterogeneity of the fish industry.

4.2 PRETREATMENT ASSESSMENT

For these by-products whose destination will be ideally human consumption again after its revalorisation, hygiene regulation for fish products is applicable. Regulation EC no.852/2004 on the hygiene of foodstuffs, includes:

- Setting out general and specific hygiene requirements.
- Developing a hazard analysis and critical control points (HACCP).
- Implementing a traceability system.

It is recommendable for all by-products, an immediate refrigeration step when generated/collected, in order to avoid degradation until they can be deep frozen.

The specific conditions for conservation and sending are:

- Shrimps: whole cooked non-compliant shrimps, frozen in 5 kg box.
- Cephalopods, cod, hake: portions, little crumbles and sawdust, frozen in 5 kg box.
- Surimi: cooked surimi roll, frozen ideally in 5 kg plastic bags.

5. REFERENCES

1. FAO. Food loss and waste in fish value chains. *FAO.* [Online] https://www.fao.org/flw-in-fish-value-chains/value-chain/en/.

2. Bachis, E. IFFO. *By-product.* [Online] 2024. https://www.iffo.com/by-product.

3. Anfaco-Cecopesca. *Complejo mar-industria alimentaria.* 2023.

4. Regulation (EC) No 1069/2009 of the European Parliament and of the Council of 21 October 2009 laying down health rules as regards animal by-products and derived products not intended for human consumption and repealing Regulation (EC) No 1774/2002. *EUR-Lex.* [Online] 21 10 2009. https://eur-lex.europa.eu/eli/reg/2009/1069/oj.

5. Myhre, M., Richardsen, R., Nystøyl, R., Strandheim, G. *Analyse marint restrastoff 2022.* s.l. : SINTEF Ocean AS, 2023.

6. Jaudzems, G., Fuerer, C. *Determination of Total Amino Acids in Infant Formulas, Adult Nutritionals, Dairy, and Cereal Matrixes by UHPLC-UV: Interlaboratory Validation Study, Final Action 2018.06.* s.l. : Journal of AOAC International, 2022. 105(6), 1625-1639.

7. Drvodelic, N. *Simultaneous determination of hydride and non-hydride elements in fish samples using the Agilent 5110 SVDV ICP-OES with MSIS accessory.* Melbourne, Australia: 5 : s.n., 2017.

8. Li, H., Gidley, M., Dhital, S. *High-amylose starches to bridge the "fiber gap": development, structure, and nutritional functionality.* s.l. : Comprehensive reviews in Food Science and Food Safety, 2019.

9. Aspevik, T., Oterhals, A., Rønning, SB., Altintzoglou, T., Wubshet, SG., Gildberg, A., Afseth, NK., Whitaker, RD., Lindberg, D. *Valorization of Proteins from Co- and By-Products.* s.l. : Springer International Publishing Switzerland, 2017. 375:53 DOI 10.1007/s41061-017-0143-6.