PROPOSAL 1

THE GOOD FOOD INSTITUTE

Sustainable Seafood Initiative Research Proposal

Detailed compositional and structural characterization of seafood products

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Executive summary

Our oceans hang in a precarious balance. Overfishing and harmful fishing practices have damaged fragile marine habitats, destabilized ocean ecosystems, and severely depleted global fisheries. The aquaculture industry has expanded rapidly as wild fisheries have collapsed, but these systems often present unique risks and limitations. New approaches are urgently needed to meet the increasing global demand for seafood without further jeopardizing aquatic ecosystems or placing undue burden on other global resources.

Plant-based and cell-based seafood present novel solutions to address these challenges by presenting consumers with more sustainable, healthier, and more humane options without compromising on taste. In the last decade, the market has seen massive shifts in consumer demand and product innovation for alternatives to meat and dairy products. These trends are likely to reflect a similar forthcoming transformation within the seafood industry, and the rapidly growing unmet demand for seafood coupled with the looming collapse of many global fisheries is likely to accelerate this shift.

However, virtually no dedicated funding outside of a few companies' R&D budgets has been expended in the development of plant-based and cell-based seafood thus far, resulting in substantial knowledge gaps for new product development. This industry exhibits tremendous potential to benefit from concerted resource allocation toward developing publicly accessible data to guide innovators in this space.

One area of urgent need is information on the parameters that define high-quality meat from a number of seafood-relevant species. A deep understanding of the molecular and structural signatures that define consumer experiences like taste, aroma, and texture is critical for developing both plant-based and cell-based products that recapitulate these sensory experiences as well as nutritional, aesthetic, and processing qualities. While the terrestrial meat industry (beef, poultry, pork, etc.) has a long history of publicly funded meat science research, detailed molecular and structural characterization of seafood products are either nonexistent or must be laboriously scraped from the scientific literature. In many cases, the data that exist are inconsistent, use outdated methods, or are simply too disaggregated to meaningfully use for guiding product development.

This proposal first establishes the parameters that define various types of seafood and surveys the data that exist on these attributes across various species. What follows is a detailed research plan for generating data to fill the knowledge gaps and a framework for incorporating both existing and novel data into a publicly accessible database.

The proposal identifies specific partners (companies, institutions, or specific research labs) with the appropriate expertise to conduct the work. Fifteen exemplar species representing several classes of seafood-relevant aquatic organisms are suggested, and three scopes of work are proposed to reflect a range of possible budgets. A "1x" scope with a budget of approximately $30,000 and a 12-week project timeline represents the minimum work that will provide meaningful results to advance the industry. Work packages corresponding to five-fold and ten-fold higher budgets are also presented, along with several optional work packages. The project scopes outlined in this proposal should be viewed as examples from a menu of possible options. Some costs scale linearly with species number and are therefore purely variable costs, but most work streams within the project present savings for higher volumes. Thus, funders are at liberty to define the scope of work such that it aligns with their mandate and mission.

The proposed research will address a critical knowledge gap that is hampering the development of high-quality, sustainable plant-based and cell-based seafood products: namely, detailed characterization of the seafood products that these approaches aim to emulate. The resulting public resource will enable researchers and innovators to accelerate the development and widespread commercial adoption of plant-based and cell-based seafood.

The transition to plant-based and cell-based seafood can be further accelerated by concerted efforts to apply insights from the development, commercialization, and generation of demand for plant-based and cell-based versions of terrestrial animal agriculture products. While many of these insights can be translated directly to plant-based and cell-based seafood, the seafood sector does pose some unique technical challenges for both plant-based and cell-based approaches. Consumer research providing a more nuanced understanding of seafood purchasing behavior across diverse consumer segments and cultures is also needed, to enable refinement of marketing and product development strategies.

While plant-based and cell-based seafood products will ultimately be produced and supplied through the private sector, the underlying technologies and their path toward commercialization will require a robust innovation ecosystem. Given that virtually no dedicated funding outside of a few companies' R&D budgets has been expended in this area and that the estimated *total* global R&D expenditure to date across all forms of plant-based and cell-based seafood is on the order of $10-20 million, this industry exhibits tremendous potential to benefit from concerted public and private resource allocation. To accelerate the process from early product development through to widespread market adoption, activities must be coordinated across startup companies, multiple sectors of established industries, private and public funders and investors, governments, trade associations, and academic and other research institutions.

All of these entities – and any individual who envisions a future with sustainable oceans of abundance – should consider this a call to action to contribute to the development and growth of the plant-based and cell-based seafood industry.

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1 Significance

Significant interest has arisen in the development of seafood products using plant-based or cell-based meat technologies. To accelerate progress, a public domain database covering the properties of seafood species pertinent to their recapitulation is needed. Certain types of high-quality information — such as nutritional data — is readily available and collated in open-access databases. Some information, such as sensory data, is either unavailable or cannot be readily compared between publications, and so would need to be created for it to have broad utility. Other information, such as seafood muscle histology, falls somewhere in between. For example, data might be available but are scattered throughout the academic literature or lack thorough standardization of methodology, and it would be preferable to generate fresh data sets using well-defined methods.

Attributes of importance to the definition of a food product include nutrient composition, sensory properties, and ingredient performance. Seafood is seen as a source of protein, fat-soluble vitamins, and omega 3 fatty acids. The sensory properties define the subjective acceptability of a food, so seafood analogs need to recreate the appearance, texture, aroma, and taste of the products they are replacing. Depending on how a product is to be used, properties related to food processing, functional properties, preparation, storage, and cooking might also be important to recreate.

Throughout this proposal, the cost versus benefit of having the different types of information has been explicitly considered. For example, sensory data is costly to generate, and despite being pertinent and interesting, it is expensive to use on a routine basis. Conversely, histology is inexpensive to generate and very useful for structural design, so falls at the opposite extreme. This proposal will outline what data are already available in the public domain, where knowledge gaps exist, and a research plan to address these gaps. The resulting data will be easily accessible to researchers and innovators to accelerate the development and widespread commercial adoption of plant-based and cell-based seafood.

2 Identification of important attributes and existing data

This section will highlight the key attributes, measurements, and considerations for providing a comprehensive characterization of seafood products from a molecular and structural perspective. A survey of the literature was conducted to identify which of these data currently exist in the public domain as a means of identifying knowledge gaps and informing the proposed work plan described in Section 3.

2.1 Preparation

When evaluating the properties of cooked seafood, it is important that the preparation method be standardized across the different types of measurement as well as being reflective of how the product is typically prepared by consumers or chefs. Different types of seafood products demand different sample preparation. For fatty fish species, it is important to consider whether the samples should be tested with or without the skin [1]. Methods need to be standardized before conducting any of the measurements on cooked seafood. Existing data in the literature are often difficult to compare due to the range of methods used in their preparation. For some species, measurements of both raw and cooked versions of the meat are necessary — for example, for species that are consumed as sushi or sashimi in addition to other preparation methods.

2.2 Yield and nutrient composition

Yield ratio of edible portion to total mass is important economically as it influences the effective price of any food. This information helps to provide an accurate cost comparison to plant-based or cell-based products, for which the yields are effectively 100% due to the absence of inedible parts such as internal organs, fins, bones, etc. As fish are consumed in different ways, the definition and numerical value of edible yield can vary significantly. For croaker fish, for example, yield has been described as mincemeat, edible portion without head, and edible portion with head, with values of 37%, 58%, and 75.5% of the total fish weight, respectively [2].

Proximate analysis provides compositional breakdown of major components including moisture, ash, and the macronutrients (protein, fat, carbohydrate). As well as providing a high-level nutritional profile, this information could be used to estimate the ingredient cost of creating a plant-based analog from a basic mass balance. It is known, however, that the lipid content of fish can vary tremendously. For example, within a single catch of herring, lipid content has been found to range from 1% to 25% [3].

Amino acid distribution looks more closely at the protein composition. This is particularly important for assessing the protein quality. The PDCAAS value (Protein Digestibility Corrected Amino Acid Score), for example, is determined from the limiting amino acid and protein digestibility. Seafoods generally have high protein quality scores, so this information is of only minor practical value. For recapitulation of seafood products, however, the amino acid distribution of the available plant proteins and their digestibility would allow the PDCAAS values of protein blends to be estimated and thereby optimized. The distribution of fatty acids from components such as triglycerides and phospholipids provides information relevant to nutritional quality and flavor, and this lipid profile should be recapitulated in plant-based and cell-based seafood products. Consumption of omega 3 fatty acids, including EPA and DHA, has been correlated with positive effects on human health such as reduced risk of some cardiovascular diseases [4] and cancers [5], and improvement of various organ functions [6]. For this reason, the FAO recommends that the ratio of omega 3 to omega 6 fatty acids in the diet be higher than 0.2 [7]. Seafood typically has a high n-3/n-6 ratio thereby enhancing the ratio in the diet as a whole. Croaker fish, for example, has a n-3/n-6 ratio of 3.1 [2].

Freshly-caught marine fish contain low levels of volatile compounds and are therefore nearly odorless [8]. Fatty acid breakdown products due to endogenous enzymes (e.g. lipoxygenase) contribute pleasant fish flavors (seaweed, cucumber, metallic, neutral). The specific fatty acid distribution of the species thereby contributes to the characteristic flavor profile. On the other hand, microbial oxidation of fatty acids can result in quality loss upon storage. Fatty acid composition is typically measured by derivatizing lipids to fatty acid methyl esters (FAME) followed by GC analysis. The fatty acid composition and total lipid content can vary greatly between species.

Micronutrient composition includes the vitamin and mineral content. This information is mainly of importance from a nutritional perspective, however, some of these components influence the taste, take part in chemical reactions, or are of importance to the molecular environment in which changes occur during cooking. Minerals are quantified by spectroscopic measurement on an ashed sample and can be measured using a single analysis. Vitamins, on the other hand, are a diverse array of chemicals that require several methods for complete analysis.

All of these basic composition parameters are commonly measured attributes of food. As a result, the measurements have become highly standardized and an industry of analytical labs has emerged that routinely perform them on a fee-for-service basis. Due to the importance of this information for the assessment of nutritional quality of diets, a large body of data is available in the public domain, mostly published by national governments and international NGOs. The United States Department of Agriculture (USDA) has a freely accessible database [9] with comprehensive nutrient composition data on many types of seafood including all species listed in Table 2. Over 260 entries currently exist in the "Finfish and Shellfish" category and there are several more under the "American Indian/ Alaska Native" category. The FAO/INFOODS uFiSh database has information on almost 70 seafood species prepared in various ways [10]. As data collected for these databases is in the public domain, exists on most food-relevant seafood species, uses highly standardized methods, and is generated by reputable labs, there is no need to duplicate this work unless funding is available to enable an exhaustive study.

Table 1: Preferred methods for compositional analysis

Component	AOAC Methoda
Amino acid distribution	Tryptophan (988.15), methionine and cystine (994.12), hydroxyproline (990.26) and all others (982.30)
Ash	923.03, 942.05, or 945.46
Fatty acid distribution	996.06
Minerals	984.27
Moisture content	950.46
Protein	968.06, 992.15, 990.03 or 991.20
Total carbohydrates	By difference from the sum of water, protein, total lipid, and ash.
Total lipids	922.06, 925.12, 989.05, 954.02, 983.23 or Folch et al 1957

[a]According to [11]

2.3 Protein digestibility

Protein quality is a function of both the limiting amino acid and the protein digestibility. These two parameters are used to calculate the Protein Digestibility Corrected Amino Acid Score (PDCAAS) value, the most widely accepted protein quality test. However, some jurisdictions use other scores such as the Protein Efficiency Ratio (PER) in Canada. In 2013, the FAO began recommending a move to the Digestible Indispensable Amino Acid Score (DIAAS) [12], which measures small intestine digestibility. In the U.S., the PDCAAS is not required for commercialization of a product, but must be known before a protein content claim is made or percent daily value (%DV) can be labelled.

Protein digestibility is usually measured via rat feeding studies using AOAC 991.29 [13], as recommended by the FAO/WHO [14] and accepted by the FDA. Recently, an *in vitro* test kit (K-PDCAAS) has been commercialized by Megazymes (www.megazyme.com), which results in the Animal-Safe Accurate Protein Quality Score (ASAP-Quality Score Method) [15]. Although this assay is not yet accepted by U.S. regulatory authorities for making claims about protein content or daily value requirements, it provides a less costly, animal-free screening tool which has been shown to agree well with the rat assay value.

Protein digestibility values have been established for many common ingredients but they may need to be determined for novel plant proteins. Protein digestibility and PDCAAS are likely to be highly useful for the commercialization of novel plant proteins and for the optimization of complementary proteins as seafood products generally have high protein digestibility and quality scores. These data are less important for existing seafood products for which the protein digestibility is either known or can be estimated. As a result, this measurement is only recommended for the 10-fold budget in order to ensure a comprehensive data set.

2.4 pH

In seafood and its analogs, pH impacts bacterial growth and thus is significant from the perspective of food safety and food manufacturing process design. It is also an indicator of sour taste. It is known that nutritional status, stress, and exercise of the fish prior to death influences the pH of their flesh, but no database is known to exist that includes pH of seafood species under various conditions. Because pH measurements are simple and inexpensive to perform, they should be performed for this project.

2.5 Aroma

Aroma is the perception of volatile chemicals by the nose and is often the major sensory differentiator between seafood species. For many seafood products, lipid-derived compounds, such as unsaturated aldehydes, alcohols, and ketones, are character-impact compounds, while methional, dimethyl disulfide, 2-acetyl-1-pyrroline, 3-methylbutanal, and alkylpyrazines are important compounds formed in cooked fish, *via* the Maillard reaction [16].

In order to evaluate aroma composition, it is necessary to extract, concentrate, separate, identify, and quantify the volatile chemicals generated by a food, as well as determine their sensory impact. Many approaches have been developed for this process. Ideally, the extract should contain the volatile components in the same relative proportions as the food itself [16]. However, due to data artifacts related to the chosen analysis approach — and because substitutions of chemical compounds are often necessary due to their availability, cost, and regulatory status — duplication of a natural flavor is usually achieved by a highly skilled flavorist using instrumental data as a starting point. The preparation (cooking) method should be carefully considered as this can significantly influence the aroma profile.

The standard technique to evaluate aroma composition is to use solid phase microextraction (SPME) to extract the volatiles from the headspace above a sample. The compounds are separated by gas chromatography and measured via a combination of flame ionization detection (concentration), mass spectroscopy (identification), and olfactometry (intensity). Olfactometry uses a human volunteer's sense of smell to determine the odor's intensity, character, and duration [17]. The data collected from each seafood species should yield a list of aroma active compounds, each with their retention time, tentative structure, relative concentration, aroma intensity, and aroma character. This would be done for each seafood species in the raw and cooked state.

The chemical compounds that contribute to aroma have been studied for many fish species. There are several examples in the academic literature of studies where a number of species have been evaluated using the same technique. For example, Morita *et al.* [18] used simultaneous distillation/extraction (SDE) with dichloromethane to examine the volatile compositions of 16 different saltwater and freshwater fish species, including tuna, cod, carp, swordfish, mackerel, eel, and flounder. Mansur *et al.* [19] used SPME to evaluate volatile flavor compounds in sea bream, chum salmon, mackerel, sardine, tuna, prawn, and shrimp. Wang *et al.* [20] used a variation of SPME in which in-fiber derivatization enabled the measurement of low molecular mass aldehydes in raw pollock. Although significant data already exist in the public domain, there is value in generating data using a common approach on all of the fish species outlined in this proposal.

The type of flavor analysis described above is routinely performed by flavor houses, many of which have dedicated teams with significant experience working directly with flavorists to duplicate natural flavors. However, such companies are typically reluctant to share data. Several academic and service companies also specialize in this area of research.

2.6 Histological observations

The texture of food is largely governed by its microstructure and the mechanical properties of the structural components. Histology is the study of the microstructure of biological tissues. Significant literature exists on the microstructure of sea animal muscle such that the information needed to recapitulate their essential features could be collected through literature review alone. Biology-oriented literature exists related to single species (e.g. [21] and [22]). Several books provide overviews on the histology of whole classes of animal e.g. [23] and [24]. These texts tend to cover the histology of all tissues, whereas food-oriented literature [25] is usually focused only on muscle tissue structure.

Despite the volume of information available, there is no single resource compiling the muscle structure of marine species frequently used as food. Additionality, differences in sampling technique, staining, image quality, magnification, etc., make comparisons challenging. To fill this gap, new images should be generated using a standardized technique. This type of work is routinely performed by histology labs in university medical departments, which are often run as a low-cost service. Computational image analysis can greatly enhance the value of microscopy results by providing quantitative data.

Quiles *et al.* reviewed techniques for studying muscle food structure [26]. Bright field microscopy with selective staining (e.g. with Mallory's trichrome) is arguably the most useful technique as it can provide information on the size and arrangement of muscle fibers. Scanning electron microscopy (SEM) provides structural information across a very broad range of magnification from macroscopic to nanoscale. As only the surface of the sample is observed, there is no need for sectioning. Cryo-SEM allows the sample to be observed without removing the water, thereby reducing experimental artifacts. Transmission electron microscopy (TEM) is used for evaluating tissue structure at the nanoscopic scale but requires more involved sample preparation [25]. As such high-magnification images are of minor importance from a food texture perspective, TEM is a less useful technique for the purposes of this project.

Fish muscle is striated, consisting of myotomes one cell deep arranged in concentric circles separated by collagenous dividing lines (myocommata). On heating, the myocommata are broken down, releasing the myotomes and resulting in the characteristic flakes of cooked fish fillets [27]. The length and diameter of the cells are important features governing the structure and resulting texture. When comparing hake to herring, for example, the main difference in the fibers is their size: hake fibers are thicker [25]. Muscle tissue from other sea creatures beyond finfish can exhibit quite different morphologies, such as squid, which have complex arrangements of orthogonally- and radially-aligned fibers, and shrimp, which are also ordered [25, 2].

2.7 Color and appearance

The first assessment of a food is often its appearance. In salmon, for instance, the intensity of red color is an important factor governing its perceived quality. Non-instrumental qualitative methods have been developed including the SalmoFan ruler (DSM) and the Natural Color System NCS® [28], and average color is easily measured quantitatively by colorimeters and spectrophotometers [29]. As instruments provide reproducible data but differing absolute values, in order to be able to compare such measurements it is important that the instrument be standardized along with sample geometry and other salient variables.

Very few comparisons across fish species have been published. The color of fish minces from different species has been measured towards the development of a grading method [30]. Studies have been published on the influence of high-pressure treatment on the color of fillets from redfish, cod, rainbow trout, whiting, haddock, and salmon [31] as well as pollock, cod, tuna, mackerel, salmon trout, carp, plaice, anglerfish, and octopus [32]. The effect of cooking has been studied on aquacultured fish fillets from pacu, rainbow trout, hybrid striped bass, catfish, and tilapia [33].

For some seafoods, the macroscopic distribution of color is also important — for example, the white lines characteristic of salmon flesh. Using either a basic flatbed scanner [34] or digital camera [35], it is possible to measure both colour and macroscopic morphologically of food products. The recommendation for this project is to generate new data on the average color (using a colorimeter) and the distribution of color for all the highlighted seafood species, both in raw and cooked states using a digital imaging technique, applying quantitative image analysis, and converting data to $L^*a^*b^*$ units.

2.8 Sensory

Sensory evaluation captures the aesthetic qualities of a food, i.e. appearance, odor, taste, and texture. Descriptive analysis (also known as sensory profiling) is the most relevant type of sensory measurement for this project. Standard methodologies exist including Sensory Spectrum, Quantitative Descriptive Analysis (QDA®), and ISO methods. This type of analysis uses familiar terms to provide a holistic picture of how a food is sensed immediately prior to and while eating. Furthermore, descriptive analysis aims to provide a quantitative, repeatable, and objective evaluation. However, as it typically involves highly trained panels, it tends to be expensive to perform on a routine basis. Also, it is challenging to fully duplicate results between panels.

Recently, to meet the need for faster turn-around and lower cost, some rapid descriptive methods have been developed, such as napping & sorting. These require less panelist training time and are especially useful when comparing many samples. As such, they may offer value for this project. The number of samples for each session depends on the number of attributes and the type of the sample. Typically, within a single session 10 attributes are measured on six samples. The amount of seafood prepared for each assessor is typically 30–100 g [1].

A number of studies have been published which have aimed to broadly evaluate differences between the sensory properties of seafood species. For example, Erickson *et al.* compared 10 different species of frozen shrimp [36], the sensory characteristics of oysters, clams, and cultured and wild shrimp have been compared [37], and sensory quality criteria have been developed for five fish species [38].

In one study, 17 fish species were characterized with the aim of creating a standardized methodology for evaluating the flavor, texture, and appearance of finfish [39]. The species clustered in three major groups: low fat, low flavor, white-fleshed fish (e.g. pollock); high fat, high flavor, dark-fleshed fish (e.g. Atlantic mackerel); and swordfish. Another study compared the aroma characteristics of 16 fish species by sensory evaluation and gas chromatographic analysis [18]. Four groupings were observed on a principal component analysis biplot around the descriptors green, fishy, fried chicken, and canned tuna.

The sensory quality of finfish and shellfish can vary considerably according to factors such as season, fishing ground, farming conditions, stress, and harvesting conditions [1]. Aquacultured fish are more predictable, but their qualities depend on factors such as feed composition, environment, fish size, and genetic traits. For example, Atlantic halibut fed a diet with different fat levels showed that larger fish had a fresher, more acidic flavor and a more juicy consistency [40]. The timing of processing and cooking of fish following death can also influence texture. Fish cooked before rigor is soft and pasty, but firm, succulent, and elastic if processed and cooked post-rigor [1]. The temperature, time, and style of cooking will also influence texture. As the goal of this project is to provide targets to aim at in the recapitulation of seafood properties, the recommendation is, as far as possible, to choose conditions for optimum quality. As optimum conditions are quite species-specific, an initial workstream in this project will develop standard protocols for handling, cold storage for transportation, and cooking samples prior to analysis.

2.9 Instrumental texture

Sensory texture is an extremely complex phenomenon related to the assessment of mechanical structure and its breakdown during mastication as detected by signals from jaw muscles, ears, and nerves on oral surfaces such as the tongue. Complicating matters further, food in the mouth changes in a number of ways including particle size, temperature, addition of saliva, bolus formation, etc. Instrumental texture measurement evaluates mechanical properties related to sensory texture but the results are rarely directly associated or correlated with sensory texture parameters. Examples of publications that do correlate instrumental and sensory texture measurements on fish do exist. One measured both fish fillets and minced fish [41], and another related blade and shear cell measurements to the sensory texture of rainbow trout fillets [42].

Many instrumental methods have been developed for measuring the textural properties of fish. They are classified as fundamental (rheological properties), empirical (correlated with sensory), and imitative (resembling actions of interest). The latter two are the most frequently reported classes of measurement on fish [27]. Examples of mechanical test systems that have been applied to the measurement of fish texture include the Kramer shear cell, Warner–Braztler blade & Fish Shearing Device (FSD), puncture, tensile and compression tests, texture profile analysis (TPA), and viscoelastic methods such as stress relaxation, creep, and small amplitude oscillatory measurements.

When using a force-deformation instrument, often the maximum force exerted during the test is measured [27]. In raw fish, there are often two peaks: the first is attributed to the muscle fibers and the second, which is sharper and larger, to the connective tissue. Sample orientation has an effect on the test when fish fillets are analyzed. Measurements made perpendicular or parallel to the orientation of the muscle fibers are regarded as estimations of fish firmness or cohesiveness respectively. A mathematical model has been developed to describe the mechanical properties of raw and cooked fish [43].

Compared to sensory analysis, mechanical property measurement is relatively inexpensive to perform, repeatable, and provides rapid feedback regarding properties which may relate to the sensory parameters of interest. These measurements are particularly valuable when the property that governs an important textural attribute can be described mechanically and an allegorical instrumental measurement can be developed. Textural analysis is most successful in the assessment of non-destructive properties, such as softness akin to pressing with a finger, or relatively simple destructive events like the force required to cut with a knife or first-bite hardness. For this project, it is recommended that tests be utilized which imitate these three actions. Mechanical measurements tend to be less successful at assessing textural properties further in the mastication process.

2.10 Properties of myofibrilar proteins

Seafood flesh is often comminuted and processed into a variety of products such as fish sticks, fish balls, fish paste, and surimi. For these products, the gelling, emulsifying, water holding, and solution properties of the protein fraction are often important. Creating a fish protein using cell-based meat technology or a plant-based protein that functions like fish protein will likely be less challenging than recreating the sensory experience of intact-muscle foods. Therefore, understanding the properties of fish proteins will be useful, especially in the short term.

Myofibrillar protein content and its solubility are of interest because oil emulsification and gelation are mostly properties of myofibrillar proteins in solution. Emulsion capacity is important for products containing a fat phase and can be determined by mixing a protein solution with a high speed rotor-stator homogenizer and adding oil until phase inversion occurs [2].

The functional role of protein in many food products is to provide a structure for holding water. Water holding capacity of fish muscle can be determined by weighing before and after centrifugation. Gel properties are arguably the most relevant to the function of fish protein in processed fish products. Gel temperature informs how the protein will perform in thermal processes, and gel strength is an indicator of its texture.

3 Proposed work

The following section details a research proposal for the creation of an open-access database and the data housed within it. This covers the recommended seafood species, work packages to be included at three budget levels, and a timeline.

3.1 Seafood species selection

A very large number of seafood species are consumed as food by humans. The FAO/INFOODS uFiSh database [10] has information on almost 70 seafood species but covers only those that are most broadly eaten. To reduce the number of species for measurement, the approach taken for this project has been to group according to class (fish, crustacean, mollusc, etc.) and subgroup (bivalves, gastropods, cephalopods, etc.) and then choose a reference species. In cases where the subgroup represents high consumption volume and significant species differences exist, multiple reference species have been chosen.

Table 2: Seafood types and recommended exemplar species for conducting these analyses

Class	Subgroup	2010 production (kT)	Examples	Exemplar species
Fish	Marine Pelagic - Predator	33,974	Shark, Tuna, Marlin, Swordfish, Mackerel	Yellowfin Tuna *Thunnus albacares*
	Marine Pelagic - Forager		Herring, Sardines, Sprats, Anchovies, Menhaden	Atlantic Herring *Clupea harengus* Alaska Pollock *Gadus chalcogrammus*
	Marine Demersal	23,806	Cod, Flatfish, Grouper, Stingrays	Atlantic Cod *Gadus morhua*
	Diadromous	5,348	Salmon, Shad, Eels, Lampreys	Atlantic Salmon *Salmo salar*
	Freshwater	43,511	Carp, Tilapia, Catfish, Bass, Trout	Blue Tilapia *Oreochromis aureus* Grass Carp *Ctenopharyngodon idellus*
Crustacean	Shrimps	6917	Shrimps	Whiteleg Shrimp *Litopenaeus vannamei*
	Crabs	1679	Crabs	Horse crab *Portunus trituberculatus*
	Lobsters	281	Lobsters	American Lobster *Homarus americanus*
Mollusc	Bivalves	12,585	Oysters, Scallops, Mussels, Cockles	Atlantic Sea Scallop *Placopecten magellanicus*
	Gastropods	526	Abalone, Conch, Limpets, Whelks, Periwinkles	Red Abalone *Haliotis rufescens*
	Cephalopods	3653	Octopus, Squid, Cuttlefish	Common octopus *Octopus vulgaris*
Other	Echinoderms	373	Sea Cucumbers, Sea Urchin, Starfish	Edible Sea Cucumber *Holothuria edulis*
	Jellyfish	404	Jellyfish	Flame jellyfish *Rhopilema esculentum*

3.2 Work packages

The work for this project has been divided into distinct work packages which can be conducted largely independently of one another under the coordination of the project manager. The work packages included in the project depend on the budget available as outlined in the table below. One challenge will be ensuring that all researchers have access to the same products. This is a logistics and storage issue that also has implications for the quality of the product (e.g. freezing vs texture).

Table 3: Summary of proposed analyses

Work type	Goal statement	Existing public domain data?	Minimum Budget	5-fold Budget	10-fold Budget
Project management	Formally kick-off and close project. Set-up agreements with vendors. Ensure coordination, communication, and completion of work packages. Resolve roadblocks as necessary.	N/A	✓	✓	✓
User friendly database	Create a user-friendly database to hold data and be a resource for product developers, including dashboards and visualization tools.	N/A	✓	✓	✓
Sample distribution	Create approach for and complete purchase, storage and distribution of samples so that they are tested by all vendors in optimum condition.	N/A	✓	✓	✓
Preparation methods	Create standard protocols for handling and thermally processing samples prior to analysis.	Scattered	✓	✓	✓
Nutritional analysis	Full macro- and micronutrient composition, including amino acid & fatty acid profiles, and edible yield on all species.	Yes	✓ (from existing DB)	✓ (from existing DB)	✓ (new data)
Appearance + pH	Evaluate appearance (color and its distribution), pH	No	✓	✓	✓
Histology	Brightfield (BF) and SEM images of raw fish muscle stained to highlight structural features related to texture taken in multiple orientations and magnifications to detail structural anisotropy.	Scattered	✓ (BF only)	✓ (BF & SEM)	✓ (BF & SEM)
Instrumental aroma analysis	Aroma profile of raw and cooked seafood using GC-MS-O providing the basis for flavor duplication and formulation with flavor precursors.	Scattered, probably incomplete	✓ (lit review)	✓ (new data)	✓ (new data)
Instrumental texture analysis	Develop methods and assess bulk mechanical properties related to handling, cutting and first bite texture on raw and cooked products.	No		✓	✓
Protein functionality	Functionality as an ingredient (gel strength, emulsification).	No		✓	✓
Sensory analysis	Create a standard seafood lexicon with descriptors and reference samples. Conduct quantitative descriptive analysis on cooked & raw (when suitable).	No			✓
Protein digestibility	Measurement of Animal-Safe Accurate Protein Quality Score enabling PDCAAS calculation.	No			✓

3.3 Timeline

The following table shows a conceptual timeline for this project assuming a scope corresponding with the 10-fold budget, forecasting project completion within 14 weeks. The 5-fold budget scope would have the same total project time span, whereas the minimum budget scope could be completed in 2 weeks less time (12 weeks).

Week 0	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12	Week 14
Project kickoff	**Setup agreements with vendors**		**Project management**				
	Develop standard preparation methods						
	Arrange storage and distribution	Purchase, distribute samples					
			Histology	Quant. image analysis			
			Nutrition				
			Finalize aroma method	Aroma analysis			
			Develop texture method	Texture analysis			
			Develop appearance method	pH, color, and appearance			
			Finalize protein functionality methods	Protein functionality testing			
			Sensory lexicon	Sensory analysis			
			Protein digestibility				
	Design database, dashboards, and visualization tools				Design database, dashboards, and visualization tools		

4 Prospective Partners

Due to the varied types of analysis within this project, it is unlikely that a single vendor is appropriate for all of the work outlined. To minimize complexity, it is advantageous to minimize the number of vendors. One approach is to utilize a single university food science department, analytical services lab, or contract research facility as the main provider, but this may require a degree of compromise as the best providers for each service are not necessarily co-located. University food science groups with a focus on seafood include University of Maine, Oregon State University, CSIC-ICTAN Madrid (Spain), Washington State University, and Central Institute of Fisheries Education (India). Another approach is to combine an analytical service lab, such as Medallion Labs, with a lab that specializes in sensory and aroma.

Table 4: Suggested potential partners for executing each aspect of the proposed work plan.

Work type	Capable Institutions
Project management	GFI or external consultant
User-friendly database	GFI or external consultant
Sample distribution	External consultant
Preparation methods	External consultant, food science departments
Nutritional analysis	Analytical service labs accredited with ISO 17025 (Eurofins, Medallion Labs, NP Analytical Laboratories)
Appearance + pH	Most food science labs, Medallion Labs
Histology and image analysis	Most universities with medical schools, e.g. Rutgers, Penn State, Tufts
Instrumental Aroma Analysis	Major flavor houses (Givaudan, IFF, Firmenich, Symrise) Academics (Andy Taylor: Nottingham, Graham Eyres: Otago, MaryAnne Drake: NCSU, Devin Peterson: FREC (Flavor Research and Education Center), Ohio State, Cornell, University of Reading) Instrument manufacturers: Gerstal FlavoLogic (flavologic.com), Aromalab (aromalab.de), Odournet (odournet.com)
Instrumental Texture Analysis	Allen Foegeding: NCSU Nottingham University Medallion Labs
Protein functionality	Binaya B Nayak, CIFE, Mumbai Hairong Bao, College of Food Science and Technology, Shanghai Ocean University
Sensory	Graham Eyres: Otago Grethe Hyldig: DTU MaryAnne Drake: NCSU Andy Taylor: Nottingham Sensory Spectrum Reckner's Institute for Sensory Research (ISR) National Food Lab UC Davis Kansas State Carolyn Ross: WSU
Protein digestibility	Medallion Labs, most analytical food science labs could be set up for this

5 Prospective Budget

Estimates for each work package described in the project proposal have been generated. In some cases, estimates were solicited from respected service vendors. In other cases, budgets were estimated using the time required to conduct the work and multiplying by a reasonable factor given the resources and sophistication required. Three budgets were created: minimum, 5-fold, and 10-fold. The minimum budget aims to generate the most essential information for the recapitulation of seafood products and relies heavily on the existing literature. The 5-fold budget covers all work areas except sensory analysis, which although insightful is very expensive and can be challenging to use for iterative product development. The 10-fold budget expands the species coverage and suggests additional work packages to generate new, more reliable data for nutrition and digestibility.

Table 5: Prospective budgets for three scopes of work

	Samples	Minimum Budget (15 species)	5-fold Budget (15 species)	10-fold Budget (30 species)	Optional
Total		**$29.3K**	**$163.3K**	**$292.1K**	
Project mgmt	N/A	$4K[h]	$6K[h]	$7K[h]	
User-friendly database	N/A	$4K[h]	$6K[h]	$7K[h]	
Dashboards and data visualization	N/A	N/A	$7K[h]	$8K[h]	
Sample purchase and distribution	raw	$2K[h]	$2K[h]	$3K[h]	
Preparation methods	cooked	$2K[h]	$2K[h]	$3K[h]	
Nutritional analysis	raw	$1K (existing data)[h]	$1K (existing data)[h]	$1.5K (existing data)[h]	$20K (new data, 15 species)[c]
Appearance and image analysis	raw and cooked	$5K[h]	$5K[h]	$8K[h]	
Color and pH	raw and cooked	$1.3K[f]	$1.3K[f]	$2.6K[f]	
Histology and image analysis	raw	$5K (BF)[e]	$10K (BF & SEM)[d,e]	$18K (BF & SEM)[d,e]	
Instrumental Aroma Analysis	raw and cooked	$5K (literature review)[h]	$42K (new data)[i]	$84K (new data)[i]	
Instrumental Texture Analysis	raw and cooked	N/A	$10K[g]	$18K[g]	
Protein functionality	raw	N/A	$30K[h]	$50K[h]	
Sensory Analysis	some raw, all cooked	N/A	$41K[b]	$82K[b]	
Protein digestibility	raw	N/A	N/A	N/A	$23K (15 species)[a]

[a] Based on a quote from Medallion Labs ($1508/sample, 15 samples)
[b] Based on a quote from Sensory Spectrum ($1405/sample/test + prep fees; 20 samples: 15 cooked, 5 raw)
[c] Based on a price list from Medallion Labs ($1,315/sample, 15 samples)
[d] Based on a price list from Medallion Labs ($250/hour, 20 hours)
[e] Based on a price list from Rutgers University NJ Medical School Histology Core Facility
[f] Based on a price list from Medallion Labs (pH $21/sample, color $62/sample, 15 samples)
[g] Based on a price list from Medallion Labs ($103/sample/test, sample prep = $100/hour, 30 samples)
[h] Approximated based on $100/hour
[i] Based on a quote from aromaLAB GmbH ($1380/sample)

6 Conclusion

This proposal presents a detailed project that is designed to advance the plant-based and cell-based seafood industry. This is accomplished by providing a comprehensive dataset on the fundamental properties of the most commonly consumed seafood species. Knowledge of these properties are required for the recapitulation of the nutritional, aesthetic, and processing qualities needed to make a successful food product. Furthermore, the information will be generated in an accessible, reproducible, and consistent format using open source methods that could be used to provide feedback during an iterative product development cycle.

The project scopes outlined above should be viewed as a menu of possible options. For example, a funder who has the capacity to support this work at the $500k level may want to prioritize a greater species breadth rather than the more expensive analyses, so they could choose to cover about 45 species at the "5X budget" level of analysis. Some costs scale linearly with species number and are therefore purely variable costs. This is true for well established fee-for-service analytical measurements, such as color, pH and instrumental aroma analysis. For most work streams within the project, however, there are savings for higher volumes because fixed costs — related to such things as set-up, contracting, method development, and database creation — only have to be paid once.

Above all, this project will provide a crucial step forward towards the commercialization of desirable seafood products while protecting the ocean ecosystems upon which we all depend.

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