

Omega-3 ingredient use in alternative meat and seafood products

Results of a 2023 survey of alternative protein startups and academic researchers

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Executive summary

Given the limited commercial availability of long-chain omega-3 ingredients from sources other than fish oil, we hypothesized that the omega-3 supply might represent a current or future bottleneck for the growing alternative meat and seafood industry. To understand this further, we conducted two surveys in late 2023 of alternative meat and seafood companies, academic researchers, and ingredient suppliers. We sought to understand alternative meat and seafood manufacturers' attitudes and current practices for sourcing and incorporating omega-3s into their products. Participants were also asked forward-looking questions to better understand their needs over the coming five years. This report summarizes and contextualizes respondents' answers, clarifying the current practices and future expectations for using omega-3 ingredients in alternative proteins.

Motivations for using omega-3 ingredients

Alternative meat and seafood manufacturers view health and nutrition as clear positive drivers for including omega-3s in their products, while impacts on flavor may act as a minor positive driver. Consumers' desire for products-especially seafood-that contain long-chain omega-3s, such as EPA and DHA,¹ may motivate companies to use these ingredients. A few companies, often focused on alternative terrestrial meat, expressed interest in fortifying their products with extra omega-3s. A motivating factor for some of these respondents was the desire to position their products as "premium," offering higher consumer appeal. However, the inclusion of omega-3s will depend on the availability and price of ingredients-if ingredients are affordable and plentiful, they are more likely to be widely used.

Use in current products

It is clear that the alternative meat and seafood industry is at an early stage when it comes to using omega-3 ingredients in current products. Alternative seafood companies seem to be very aware of the importance of omega-3s, though not all are yet prioritizing them in their current products. Currently, omega-3s are not a major priority for most alternative terrestrial meat companies.

Current challenges

While companies are at least somewhat motivated to use omega-3 ingredients in their products, they reported a number of obstacles that make this difficult. The main obstacle is the cost of ingredients. Additionally, omega-3s are unstable and prone to oxidation, which creates off-flavors and limits shelf life. Finally, animal-free ingredient sources of longchain omega-3s, suppliers, and supply are limited.

Ingredients of interest

Respondents expressed a clear openness to diversification of omega-3 sources. Whereas almost all currently rely on long-chain omega-3 ingredients from algae, they also expressed substantial interest in ingredients from other marine microorganisms, precision fermentation, and plant molecular farming. Encapsulation techniques that protect omega-3s from oxidation were also of interest for future use.

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¹ EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid. EPA and DHA are generally not produced in abundance outside of marine sources such as microalgae, and are the main omega-3s associated with the health benefits of seafood.





Five-year projections and future supply chain implications

Companies expect to place a greater priority on omega-3s in their products in five years compared to today. They also expect to purchase larger quantities of ingredients and to face more substantial challenges in doing so. Comparing current volumes of algal oil production with estimates of future EPA and DHA ingredient needs in the alternative meat and seafood industry indicates that these ingredients are likely to become a future bottleneck. More specifically, if cultivated seafood² uses algal oil as its primary EPA and DHA source and grows in line with third-party market projections, then foodgrade algal EPA and DHA production would need to increase by approximately 35–132 percent by 2030, and by 429–1717 percent by 2050 (relative to 2022 levels). Although we expect alternative terrestrial meat to contribute substantially less to EPA and DHA ingredient demand, it would still require a further 111–222 percent increase³ in food-grade algal oil production by 2050.

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² Because specific projections for the long-term growth of alternative seafood (including plant-based and fermentation-derived products) have not been published, we relied on cultivated seafood as a proxy for the broader category of alternative seafood.

³ This is despite the fact that the 2050 projections used here for alternative meat production are 17–34x larger (by weight) than those for cultivated seafood.

Key recommendations

The following recommendations are provided for alternative protein companies and ingredient suppliers interested in working with the alternative protein industry. Recommendations for researchers and R&D funders are derived from answers to an open-ended survey question about the main knowledge gaps related to omega-3s for alternative meat and seafood (for more detail, please see page 51).

Recommendations to alternative protein companies

For companies planning to develop EPA- and DHArich products, begin developing relationships with suppliers early on. While omega-3 ingredients may not be a challenge for many early-stage startups, the majority of respondents from companies beyond the R&D stage reported that sourcing omega-3 ingredients was at least a moderate challenge (Figure 7).

Solicit consumer feedback when considering whether <u>omega-3-enhanced products</u> are appropriate as part of your company's long-term strategy. It remains an open question to what extent this is something that people want, and it may be the case that such products are desirable in some product categories and not others.

For those struggling to find suppliers, take advantage of existing resources that aggregate this information. GFI's company database contains information about <u>ingredient and equipment</u> <u>suppliers</u> interested in working with the alternative protein industry, including omega-3 suppliers. The Global Organization for EPA and DHA Omega-3s (GOED) also maintains a filterable <u>database</u> of their member companies, which includes a number of non-animal EPA and DHA suppliers.

Lead with transparency when communicating with consumers. Specify the fatty acid profiles of your products, including distinguishing between ALA and EPA/DHA.⁴ Identify sources when possible. Designate the amount of specific key fatty acids per serving. Avoid vague claims (e.g., "high in omega-3s"), which may not be permitted by regulation.

Recommendations to ingredient suppliers

Continue to scale up production of algal EPA and DHA. Alternative protein companies are interested in EPA and DHA ingredients from multiple sources, with the largest number of respondents expressing interest in algae (Figure 12). Such ingredients have the potential to become a major bottleneck as the alternative protein industry grows (Figure 36, Figure 37, Figure 38).

Develop new ingredients from sources besides algae. Survey respondents were especially interested in EPA and DHA from other marine microorganisms besides algae, recombinant microorganisms, and recombinant plants (plant molecular farming) (Figure 12). These ingredients can complement those from algae to address the future supply gap.

Develop and market encapsulated omega-3 ingredients from the sources mentioned above. Of those companies not already using encapsulated ingredients, the majority are interested in doing so (Figure 25).

⁴ ALA: <u>α-linolenic acid</u>, an omega-3 fatty acid found in plant sources such as flax seeds. EPA: <u>eicosapentaenoic acid</u>; DHA: <u>docosahexaenoic acid</u>. EPA and DHA are generally not produced in abundance outside of marine sources such as algae, and are the main omega-3s associated with the health benefits of seafood.

Win customers by offering cost-effective products that are protected against oxidation, marketing your products to alternative protein companies, and decreasing lead times. The top five challenges when sourcing omega-3s, according to our results, are price, trouble finding suppliers, shelf life, long lead times, and off-flavors (Figure 8).

Consider the growth of the alternative protein industry as a potential business opportunity. Because the timeline of this growth is uncertain, we recommend looking at alternative proteins as one piece of a diversified strategy to mitigate risks associated with mismatches in ingredient supply and demand. Ingredient suppliers to more mature markets, such as aquafeed and nutritional supplements, can take advantage of alternative proteins as an opportunity to add a new revenue stream.

Begin developing relationships with alternative protein companies early! Considering the fact that trouble finding suppliers was identified as a top challenge, suppliers interested in working with this industry can develop a competitive advantage by being proactive in finding customers. We welcome ingredient suppliers to <u>add themselves</u> to GFI's company database.

Recommendations to researchers and R&D funders

Oxidation and stability: Develop methods for preventing oxidation and improving the stability of omega-3 ingredients, including at room temperature, as well as strategies for preventing omega-3 oxidation in finished products.

Human health and bioavailability: Continue to improve our understanding of the relationship between omega-3 intake and human health, as well as differences in the bioavailability of omega-3s depending on their chemical form, source, and the surrounding food matrix.

Diversifying sources: Develop or identify additional long-chain omega-3 sources, including by screening microorganisms to find those with naturally high levels of these compounds and by developing recombinant sources.

Life cycle and techno-economic assessments: Rigorously evaluate the environmental impacts and production costs at industrial scales for those sources deemed most promising.

Omega-3s and food formulation: Investigate methods for incorporating omega-3s into alternative protein products. For cultivated meat and seafood, this can include strategies where omega-3s are added as an ingredient in a product formulation, as well as those where they are added earlier in the cell cultivation process. As part of this work, also consider any impacts of the formulation process on omega-3 oxidation and stability and evaluate the economics of strategies where bioconversion is a necessary part of the process.

Findings at a glance

The two surveys described in this report aimed to improve our understanding of omega-3 ingredient use by alternative meat and seafood producers. They provide insights into the omega-3 content of respondents' current products, their current ingredient sourcing practices and main challenges, and their expectations of how these things will change in the coming five years. Below is a summary of our main findings.

Outlook for omega-3 ingredients in alternative meat and seafood

By comparing participants' responses to questions about the current and anticipated state of their company in five years' time, we learned the following:

- Companies expect to place more emphasis on omega-3 content (both total and EPA/DHA) in their future products compared to today (Figure 27, Figure 28).
- Companies expect needing to source substantially higher volumes (~100–1,000x, based on median responses) of omega-3 ingredients (Figure 32).
- Sourcing omega-3 ingredients is moderately challenging for some alternative protein companies and researchers today. Assuming no change in the omega-3 ingredient industry, companies expect to face greater challenges in sourcing ingredients within the next five years (Figure 33).

We also performed some calculations to better understand the relationship between the size of the alternative protein industry and its omega-3 ingredient needs. The current volume of human food-grade algal omega-3 ingredients (the main source of non-animal-derived EPA and DHA) produced per year is quite small. More specifically, if cultivated seafood were to grow in line with other organizations' near-term projections, its EPA and DHA needs alone would outstrip current algal ingredient production around 2030 (Figure 36). Thus, it seems that companies are correct in predicting that these ingredients could be a substantial bottleneck within the next five years.

EPA and DHA sources

Algae was by far the most common source of EPA and DHA used by both companies and academic researchers (Figure 12). It was also the most selected response when we asked about the sources respondents were interested in using in the future. However, there was also robust interest in marine microorganisms other than algae, recombinant plants (i.e., plant molecular farming), and recombinant microorganisms (i.e., precision fermentation), and some interest in cell-free systems (Figure 21), implying that respondents are interested in diversifying their EPA and DHA sources beyond algae alone. In their answers to free-response questions, participants identified the development of novel animal-free EPA and DHA sources as a key knowledge gap and the lack of diversity in the available non-animal EPA and DHA sources as an obstacle to wider use of such ingredients in alternative meat and seafood.

Cost and sustainability

The price of ingredients was the most frequently selected challenge related to sourcing omega-3s (Figure 8). Consistent with this, cost was also the most frequently mentioned theme in answers to a free-response question about obstacles to wider omega-3 fortification. Free-response answers also highlighted both cost and sustainability as key knowledge gaps.



Chemical forms and level of processing of omega-3 ingredients

Most respondents reported using EPA and DHA ingredients in the form of free fatty acids or triglycerides, with a substantial proportion also using phospholipids (Figure 13). Responses were broadly similar when asked what forms they would like to use (Figure 24), suggesting that ingredients largely meet users' needs in this respect. For omega-3s in general, our results did not show a clear preference between concentrates, refined oils, and minimally processed oils (Figure 26).

Oxidation and stability

Shelf life and off-flavors were within the top five most frequently selected challenges in sourcing omega-3s, respectively (Figure 8). These issues, along with oxidation, were also frequently mentioned as knowledge gaps and obstacles in answers to free-response questions.

Encapsulated ingredients can help prevent oxidation and improve stability; however, the vast majority of respondents reported not currently using such ingredients. This does not reflect a lack of interest, as around half of those respondents were interested in using encapsulated ingredients in the future (Figure 25). Thus, encapsulation or other methods for preventing oxidation may be a key white space for future omega-3 ingredients.

Omega-3-equivalent and omega-3-enhanced products

For alternative seafood companies, appealing to consumers by producing nutritionally equivalent products—in terms of both total omega-3s and EPA/ DHA—is an important motivation. Many of these companies are already targeting or achieving this goal, and almost all claimed that it was a target within the next five years (Figure 29). Several companies expressed interest in omega-3-enhanced products as a future target, though this was less common.

For some alternative terrestrial meat companies, the production of omega-3-enhanced products represents an opportunity to appeal to consumers by producing "premium" products. It is generally the case, however, that this goal is not yet being prioritized in current products. These companies varied when it came to their future targets, with some interest in both equivalent and enhanced products, and some companies not planning to prioritize omega-3s in the next five years (Figure 29, Figure 30). Most terrestrial meat-focused respondents indicated openness to the idea of omega-3-enhanced products (Figure 31).

Introduction

Omega-3 fatty acids, especially EPA and DHA, are nutritionally valuable components of seafood that are important to consumers but are not yet widely available from non-animal sources. Developing a robust supply chain for sustainable sources of EPA and DHA will be crucial to the future success of the alternative seafood industry, and may be relevant for other alternative protein products.

Alternative proteins—including those made from <u>plants</u>, <u>microbial fermentation</u>, and <u>cultivated animal</u> <u>cells</u>—have the potential to mitigate many of the <u>environmental</u> and other harms associated with animal agriculture. For this theory of change to work, these products truly need to live up to consumers' expectations. That means they need to be at least as tasty, affordable, convenient, and nutritious as their animal-derived counterparts.

Many of the health benefits associated with seafood are due to its high concentration of long-chain omega-3 fatty acids (Guo et al. 2019; Zhang et al. 2021). Omega-3 content is an important consideration for health-conscious consumers. This extends to alternative seafood, where around a third of potential consumers listed omega-3 content as at least somewhat important to their hypothetical decision to choose alternative seafood or not (Azoff 2021). Omega-3s are best known for their benefits to <u>heart, brain, eye, prenatal, and maternal health</u>. In addition, omega-3s may also contribute in some cases to the positive attributes of <u>seafood flavor</u>.

However, omega-3s come in a variety of forms, and these are not necessarily equivalent from a nutritional standpoint. Seafood is specifically known for its high concentration of long-chain omega-3s, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Some plant oils, such as flax (Kobata, Zhang, and McClements 2023), walnut, canola, soybean, and chia oils, contain high levels of the omega-3 α-linolenic acid (ALA) (NIH 2023). ALA is not naturally synthesized in animals (including humans) and is mostly stored or used for energy upon consumption. ALA can be converted into EPA and DHA, but this process is inefficient in animals (Figure 1). Fish and other marine animals that are high in EPA and DHA do not produce them directly, and instead acquire them by eating EPA- and DHA-rich microorganisms.



Legend

- ↓ Many organisms in this group can carry out this step efficiently.
- Organisms in this group may be capable of carrying out this step, but only inefficiently or in small quantities.
- Most organisms in this group are incapable of carrying out this step.

Figure 1. Schematic showing the omega-3 synthesis capabilities of terrestrial plants, marine microorganisms, and animals. The steps shown here are simplified, and in reality include the action of multiple enzymes. Created with Biorender.com.

While there is evidence for health benefits from ALA itself (Sala-Vila et al. 2022), it is not considered to be interchangeable with EPA or DHA (Jesionowska et al. 2023). As a result, omega-3 fatty acid sources rich in EPA and DHA are preferred for alternative seafood products to optimize nutritional value. With that in mind, ALA and other <u>shorter-chain omega-3s</u> from crop sources can serve as precursors for elongation by hepatocytes, engineered microbes, or cell-free methods. Omega-3 content is a less important consideration for alternative terrestrial meat, but may present opportunities to improve alternative protein products' nutritional value.



Survey participants

The main survey (survey 1) was targeted toward representatives of alternative meat and seafood companies, as well as academic researchers who use omega-3 ingredients in their alternative protein-related work. We also integrate results from several questions that were included in GFI's annual survey of the alternative protein industry (survey 2).

Most survey 1 respondents (84%) represented early-stage alternative protein companies, with some contributions from academics and more mature companies. Most respondents focused on either cultivated or hybrid proteins, with a substantial number focusing on plant-based products and a smaller number on fermentation. Seafood—especially salmon—was the main product target, with only a few respondents working on terrestrial alternative meat products, and a substantial minority focusing on both seafood and terrestrial products. In comparison to survey 1, survey 2 respondents were more likely to represent mature companies and those focused on terrestrial alternative protein products.

Respondent data

Both surveys⁵ first asked some basic questions about the respondent or their company and their role in the alternative protein ecosystem. The majority of survey 1 respondents represented alternative protein companies, while a few were academic researchers (n=31, Figure 2A). In total, twenty survey 2 respondents (out of 171 given the option) answered at least one of the seven questions about omega-3s. Most were alternative protein companies, and just over a quarter were suppliers (Figure 2B). Respondents representing companies (in survey 1; n=26; Figure 3A) or all respondents (in survey 2; n=20; Figure 3B) were asked to indicate the stage at which their company operates. Because no specific definitions were given in the question, it is important to note that respondents classified themselves according to their own understanding of these terms, which may have differed somewhat from person to person. Indeed, results from another recent survey revealed substantial differences in how companies define the stage of their operations (Harsini and Swartz 2024). Therefore, the distinction between, for example, "R&D" and "Prototype" may be somewhat fuzzy. Even so, distinctions such as that between "R&D" and "Industrial scale" can be assumed to be reliable.

Different terminology was used to describe production stages in the two surveys, so an exact 1:1 comparison is not possible. However, responses to survey 2 generally came from more mature companies (i.e., those operating at demonstration or commercial stages) compared to survey 1.

⁵ See Appendix for survey details and methodology. Unless otherwise stated, results throughout this report can be assumed to come from survey 1.

Respondent type (alternative protein company, researcher, or supplier)



Figure 2. Types of individuals or organizations responding to survey 1 (A) and survey 2 (B). AP = alternative protein. A: Q7. Which of these best describes you/your company? B: Q2-2. Please select your organization type. If you fall into more than one category, please select the one that best describes your organization. Q2-3. Which of the following primarily describes your company?



Company stage

Figure 3. Company stage of respondents to survey 1 (A) and survey 2 (B). A: Q11. How would you describe the stage at which your company is currently operating? B: Q2-25. In which production stage is your company currently operating?

Product data

Respondents were asked to indicate which type of alternative protein product is the focus of their lab or company. To account for the existence of hybrid products—meaning those with some combination of plant-based, fermentation-derived, and cultivated ingredients—respondents could select multiple options. Responses with multiple options selected are shown here as "hybrid." Approximately equal numbers of survey 1 respondents focused on plant-based, cultivated, and hybrid products, with a smaller number focusing on biomass fermentation (n=31, Figure 4A). While hybrid products can refer to any combination of alternative protein production methods, of the eleven respondents classified as "hybrid" in this sample, eight indicated that they worked on cultivated products. This suggests that the majority of those in this category are working on cultivated meat or seafood products that also include some percentage of plant-based or fermentationderived ingredients. Thus, the sample for this survey is slightly biased toward companies and researchers working on cultivated meat and seafood, with a substantial representation of those focusing on plant-based proteins and fewer fermentation companies and researchers.

Respondents to survey 2 were most commonly focused on hybrid or plant-based products, closely followed by cultivated products (n=20, Figure 4B).



Alternative protein production method

Figure 4. Alternative protein production methods used by survey 1 (A) or survey 2 (B) respondents. A: Q8. Type of product your company or lab focuses on. B: Q2-5. In which alternative protein category(ies) is your business involved? (select all that apply)

Respondents were asked about the species focus of their work by choosing from a pre-selected list, as well as (in survey 1 only) by entering text if they chose one of the "Other" options. For display purposes, each response was classified as either "Seafood," "Terrestrial," or "Seafood and Terrestrial," depending on whether the chosen options belonged to one or both categories. The majority of survey 1 respondents focused on seafood exclusively, with the next-largest group focusing on both seafood and terrestrial meat and a smaller group focusing on terrestrial meat alone (n=31, Figure 5A).

The overrepresentation of alternative seafood companies in survey 1-the explicit focus of which was omega-3s-is not surprising. Seafood products have a strong reputation for their content of longchain omega-3 fatty acids, and this is not generally a main focus for terrestrial meat. Even so, the strong bias toward seafood in early responses to survey 1 motivated us to add several omega-3 questions to survey 2-the focus of which was more general-to get a high-level understanding of omega-3 ingredient use in alternative terrestrial meat.

Ten survey 2 respondents listed meat, seafood, or both among the product types they focus on, with terrestrial-focused companies being more common than seafood-focused (n=20, Figure 5B). Those listing neither focus were a fairly diverse group that included companies focused on ingredients and inputs, oils and fats, and dairy, with many respondents selecting multiple options.



Focus on seafood versus terrestrial meat

Figure 5. Product focus of respondents to survey 1 (A) and survey 2 (B). A: O9. Species/product focus. For respondents who chose "Other," also includes responses to Q10. Please specify the species you're focusing on. Note: Any specific responses to Q10 were manually aggregated before presenting the data. B: Q2-6. Company focus: (select all that apply)

For a more detailed look at survey 1 respondents' species focus, we also separately plotted the number of times each option was selected (Figure 6). This revealed that salmon is the most common product focus, followed by a four-way tie between other finfish, shrimp, chicken, and tuna. This level of granularity was not included in survey 2, where participants were simply asked whether their products included alternative meat or alternative seafood.



Focus on specific products or species

Figure 6. Alternate view of survey 1 respondents' species focus based on responses to Q9-10. Counts represent the number of times each option was selected, and a given respondent may be counted more than once. Bars for "Other finfish" and "Other terrestrial animal" reflect instances where the response to Q10 was a general category such as "freshwater fish." These counts do not include more specific responses such as "trout" or "eel."

Current state of omega-3 ingredients for alternative meat and seafood products

With the exception of companies at the R&D stage, most respondents reported that sourcing omega-3 ingredients currently represents a substantial challenge but not a prohibitive one. More specifically, the main challenge is the price of ingredients, followed by trouble finding suppliers, shelf life, long lead times, and off-flavors.

Respondents almost exclusively purchase omega-3s rather than producing them in-house, and most are currently purchasing omega-3 ingredients in small volumes (10–100 kg per year or less). Most of the ingredients purchased have low (<10%) or unknown omega-3 content, though there was substantial variation, with some respondents reporting the use of ingredients with up to 60–70% EPA/DHA and total omega-3. EPA and DHA were sourced mainly in the form of free fatty acids and triglycerides, with algae being by far the most commonly used source. Very few respondents reported using encapsulated omega-3 ingredients.

Are omega-3s a challenge and why?

We asked survey 1 respondents to indicate how big of a challenge sourcing omega-3 ingredients—including EPA and DHA—currently represents for their lab or company. The following definitions were provided as part of the question:

Minor challenge

Sourcing these ingredients introduces occasional challenges or headaches (e.g., prices are higher than we would like, or we sometimes run into delays with orders).

Medium-size challenge

Sourcing these ingredients introduces substantial issues (e.g., prices are high enough to substantially affect the cost of production, or we sometimes have to delay production runs or experiments due to issues with ingredient orders, but these issues are not prohibitive).

Major challenge

Issues with sourcing these ingredients seriously impacts our ability to conduct our experiments or to produce products at the scale, price, and quality that we otherwise could. The most frequent response was that sourcing omega-3s is a "Medium-size challenge," followed by "Not a challenge" (n=25, Figure 7). It is worth noting that company responses differed substantially depending on company stage. When excluding responses from R&D stage companies, the "Mediumsize challenge" option stands out as by far the most common response. This suggests that sourcing omega-3 ingredients introduces substantial though not prohibitive—issues for most companies once they reach a certain point in the product development process. Most academic researchers classified it as a minor challenge, while one said it was a major challenge.

How big of a challenge is omega-3 sourcing today?



Figure 7. Q16. How big of a challenge would you say sourcing of omega-3s, including EPA and DHA, is for your lab or company currently? Colors represent company/academic status (Q7) and company stage (Q11).



One possible interpretation of the data presented here is that many early-stage companies are either ignorant of the challenges posed by omega-3 ingredients or believe-rightly or not-that this will not be a challenge for them. Out of those respondents who both classified themselves as R&D stage and answered "Not a challenge," four out of six provided additional information in response to an open-ended follow-up question. These responses made clear that sourcing or producing omega-3s is part of these companies' plans, but they are not yet at the stage where it is an active challenge. Among this same group of six companies, responses to a later question (Q45) about the anticipated magnitude of this challenge in five years varied substantially. All but two indicated that it would be at least a minor challenge (one answered "Not a challenge" and one skipped the question). Based on the data available, it seems that early-stage companies are reasonably aware of omega-3s as a future challenge.

This result indicates that some of our respondents are at an early stage where they are not yet navigating the challenges associated with sourcing omega-3 ingredients, even if they are aware of them in theory. To account for this, many of the other results in this report will be colored according to the stage of the company. Because of the small number of respondents classifying themselves as industrial, demonstration, and prototype stages, responses to the company stage question (Q11) have been collapsed from five to three categories for simplicity and to better preserve respondents' anonymity. For some questions, it may be prudent to give more weight to answers from company respondents who are past the R&D stage, as these individuals may have unique insights into the challenges associated with omega-3s.

Respondents were also asked to indicate the specific challenges they face when sourcing omega-3 ingredients. The most commonly selected responses were price, finding suppliers, shelf life, off-flavors, and long lead times (n=22, Figure 8). The two "Other" responses represented the scalability of animal-free technologies and difficulty in finding Kosher Badatz products.



Specific challenges when sourcing omega-3s

Figure 8. Q17. What specific challenges are you facing when it comes to sourcing omega-3s? (Please select all that apply.) Colors represent company/ academic status (Q7) and company stage (Q11).

Responses to Q19 reiterated that the main challenges are price and finding suppliers. Shelf life and other issues related to product quality were also mentioned. For example, one respondent wrote:

Q19. Please elaborate on the extent and nature of your challenges when sourcing omega-3-containing ingredients.

"Our experience has been that there are limited number of suppliers, high prices, and limited options for customization of the formulations."

What volumes of ingredients are respondents currently purchasing?

Most respondents indicated that they currently purchase small volumes of omega-3 ingredients, if any (n=26, Figure 9). Unsurprisingly, this is especially the case for R&D-stage companies and academics.

Volume of omega-3 ingredients (kg) purchased in the past year



Almost all respondents purchase ingredients rather than producing them in-house (n=22, Figure 10).

Are EPA and DHA purchased or produced in-house?



Figure 10. Q20. Do you purchase EPA and DHA or produce them in-house? Colors represent company/academic status (Q7) and company stage (Q11).

Most purchased ingredients have low or unknown total omega-3 (Figure 11A, n=21) and total EPA/DHA (Figure 11B, n=21). However, some respondents reported purchasing ingredients with EPA/DHA and total omega-3 content up to 60–70 percent.

No respondents reported purchasing large volumes of high-concentration omega-3s. One reported purchasing 10,000-100,000 kg of ingredients per year, but with fairly low total omega-3 and unknown EPA/DHA content.

Figure 9. Q13. How many kilograms of omega-3 ingredients has your company or lab purchased in the past year? Colors represent company/ academic status (Q7) and company stage (Q11).

Total omega-3 and EPA/DHA content of ingredients



Figure 11. Total omega-3 (A) and EPA/DHA (B) content of ingredients currently in use by survey participants. A: Q14. For the ingredients mentioned in the question above, what is the total omega-3 content? B: Q15. For the ingredients mentioned in the question above, what is the total content of EPA and DHA combined? Colors represent company/academic status (Q7) and company stage (Q11).



What types of ingredients are respondents currently using?

Survey participants were also asked about the ingredients they currently use as sources of EPA and DHA for their products. The vast majority reported using ingredients derived from algae, with smaller numbers using animal sources, recombinant microorganisms, or recombinant plants (n=21, Figure 12). The two companies who use animal sources also reported using at least one other source.

EPA and DHA sources currently in use



Figure 12. Q22. Where are your EPA and DHA sources coming from? (Please select all that apply.) Colors represent company/academic status (Q7) and company stage (Q11). EPA and DHA are sourced in a variety of chemical forms, primarily free fatty acid, triglyceride, and phospholipid (n=15, Figure 13). We also asked about the use of encapsulated ingredients. Very few respondents (3 out of 25) reported using such ingredients currently, though many are interested in doing so (see page 37).

EPA and DHA forms currently in use



Figure 13. Q26. In what form(s) are you currently sourcing EPA and DHA? (Please select all that apply.) Colors represent company/academic status (Q7) and company stage (Q11).

Current state of alternative meat & seafood products with respect to omega-3s

Most companies who responded to the survey are either targeting equivalence with conventional products regarding omega-3 (including EPA/DHA) content or not considering it in their current products or prototypes. When excluding very early-stage companies, many of whom are not yet prioritizing omega-3 content, there were roughly equal numbers in these two categories. A few companies are pursuing omega-3 or EPA/DHA-enhanced products, and report having achieved this at the prototype stage. Achieving equivalent or higher omega-3 content is not a current priority for the few company respondents who are focusing on terrestrial alternative meat products. Cultivated meat and seafood companies report using a variety of strategies to introduce omega-3s into their products, with addition to the culture media being the most common.

What is the omega-3 content of current alternative meat and seafood products?

Because the alternative protein field—particularly alternative seafood—is at an early stage, many startups are still developing their products. To contextualize responses from companies at various stages of the product development process, we asked company respondents to indicate whether their answers on the survey reflected the characteristics of their currently available products, prototypes where the fatty acid profile had already been verified, or simply the targets they were aiming for (n=22; n=26 counting companies with no response, Figure 14). Most companies were not yet on the market, and slightly fewer than half of those at earlier stages had characterized their prototypes' fatty acid profiles.

Responses to the earlier question about company stage were closely related to responses to the question about prototype or product status (Figure 15). Companies with products on the market ranged from pilot to industrial scale. Most of those companies who reported having a prototype with a fully characterized fatty acid profile classified themselves as pilot stage. Those with target profiles only, as well as those who declined to answer the question, mostly classified themselves as R&D stage.

For both total omega-3s (n=22) and EPA/DHA (n=23), the majority of companies are either targeting equivalent content or not explicitly considering it in their current products or prototypes (Figure 16). Companies without at least a characterized prototype are overrepresented among those not considering omega-3 and EPA/DHA content, though this response was still the first (for EPA/DHA) or second (for total-omega-3s) most common after excluding these companies. Few companies are targeting higher content. Those who reported targeting higher content (for both total and EPA/ DHA) were prototype- or pilot-stage companies who also reported having a prototype with a characterized fatty acid profile. This indicates that omega-3-enhanced products have been achieved at the prototype stage but are not yet on the market, at least by the companies represented in this survey.



Status of respondents' products or prototypes



Figure 14. Status of company respondents with regard to the fatty acid characterization of their products or prototypes. Q33 (presented at the start of the "Current products" section). My answers in this section reflect: ("With characterization" means that you have produced prototypes and have characterized their fatty acid profile. "Target" means that you are at an earlier stage in the product development process and are answering with respect to the fatty acid profile you're aiming for in your current prototypes, but haven't yet confirmed that this has been achieved.) "Product status unknown" indicates that a respondent is from an alternative protein company but did not provide an answer to Q33.

Product/prototype status versus company stage



Figure 15. Correspondence between self-identified company stage and product/prototype status. Q11. How would you describe the stage at which your company is currently operating? Colors represent product/ prototype status (Q33).



Total omega-3 and EPA/DHA content of current products or prototypes

Figure 16. Total omega-3 (A) and EPA/DHA (B) content of participants' current products, prototypes, or targets. A: Q35. Compared to conventional equivalents (e.g., conventional salmon if your company makes plant-based salmon), what is the total omega-3 content of your company's current products or prototypes? B: Q36. Compared to conventional equivalents (e.g., conventional salmon if your company makes plant-based salmon), what is the EPA/DHA omega-3 content of your company's current products or prototypes? Colors represent product/prototype status (Q33).

Overall, the inclusion of omega-3s seems to be of some interest to companies but may not always rise to the level of a top priority for current products. This may reflect the need to balance the benefits of omega-3s with the challenges discussed previously. For example, one respondent wrote:

"We are interested in these ingredients as a possibility to improve the nutritional profile, but the price is a major concern as well as texture & shelf stability." (response to Q43)

Plotting the same data on shared axes shows fairly little difference in the distribution of responses to the questions about total omega-3 and EPA/DHA (Figure 17).

Total omega-3 and EPA/DHA content of current products or prototypes



Figure 17. Comparison of total omega-3 versus EPA/DHA content of current products, prototypes, or targets. Q35. Compared to conventional equivalents (e.g., conventional salmon if your company makes plantbased salmon), what is the total omega-3 content of your company's current products or prototypes? Q36. Compared to conventional equivalents (e.g., conventional salmon if your company makes plantbased salmon), what is the EPA/DHA omega-3 content of your company's current products or prototypes? Notably, we saw substantial differences between alternative protein companies focused on terrestrial meat and seafood. Among companies with an exclusive focus on seafood who also reported having at least a prototype with a characterized fatty acid profile, "Equivalent omega-3 content (±10%)" was the most common response to Q35 (Figure 18). Among this same group, "Equivalent EPA/DHA content (±10%)" was the most common response to Q36, followed by "Not a consideration" and "Higher content." Those focused on terrestrial meat or both categories were most likely to answer "Not a consideration" to both questions, while seafoodfocused companies at earlier stages fell somewhere in between (discussed further on page 40).

Alternative seafood companies are more likely to target equivalent omega-3 and EPA/DHA content than alternative meat companies



Figure 18. Total omega-3 (A) and EPA/DHA (B) content of participants' current products, prototypes, or targets, colored by product status and species focus. A: Q35. Compared to conventional equivalents (e.g., conventional salmon if your company makes plant-based salmon), what is the total omega-3 content of your company's current products or prototypes? B: Q36. Compared to conventional equivalents (e.g., conventional salmon if your company makes plant-based salmon), what is the EPA/DHA omega-3 content of your company's current products or prototypes? B: Q36. Compared to conventional equivalents (e.g., conventional salmon if your company makes plant-based salmon), what is the EPA/DHA omega-3 content of your company's current products or prototypes? Colors represent species focus (Q9-10, manually aggregated) and product/prototype status (Q33) for those with a focus on seafood.

Public statements by alternative seafood companies

Some alternative seafood companies have provided information about the omega-3 content of their products on their websites or in statements to the media. Many of these statements reflect a focus on nutritional equivalence to conventional products. For example, a 2022 article about the cultivated seafood company BlueNalu refers to the goal of recreating "the exact nutritional profile of bluefin tuna toro from an animal, with omega-3s and saturated and unsaturated fatty acids." According to the website* of cultivated seafood company Wildtype Foods, "Wildtype salmon has the same amount of omega 3's and omega 6's as conventional salmon, but it's still a work in progress and we'll have many opportunities for improvement over the years to come. Achieving nutritional comparability, particularly when it comes to protein, healthy fats, and micronutrients is a key product development goal for our crew." The alternative seafood company Finless Foods-which focuses on both plant-based and cultivated products-stated in 2023 on their blog: "Finless plant-based pokestyle tuna is minimally processed, low in sodium, contains Omega-3 fatty acids, and is made of nine whole, plant-based ingredients to mimic the taste and texture of wild-caught tuna." A 2023 article about a cultivated seafood partnership between Umami Meats and Steakholder Foods stated that the companies are "working to optimize the nutritional profile to achieve the same amino acid, omega-3 fatty acid, and micronutrient profile that consumers expect from high quality fish products." A 2024 press release from the cultivated seafood company Wanda Fish stated, "The fat endows the cultivated fish whole cut with not only its velvety texture but also its unique, rich flavor and essential nutrients, including omega-3s."

Some statements specifically mention EPA and DHA or list specific omega-3 sources. For example, Good Catch's website* includes ingredient lists for their plant-based crab, fish, and tuna products. Some of these include algal oil, which is described in a footnote as a "Plant Source Of Omega-3 DHA." Similarly, Revo Foods states on their website*: "Revo™ Salmon Spread is a great source of proteins, Omega-3 fatty acids (DHA/EPA), and vitamins. Made of pea proteins, microalgae oils, and organic soymilk, it is both nutritious and delicious." The same company also published a blog post in 2023 about algal-derived omega-3s that touched on human health, flavor, and sustainability. Hooked Foods' website* states: "Smoked Salmoonish is made with protein from soy, pea and wheat which, together with algae, provides a high protein and Omega 3 content just like normal salmon." Save Da Sea was profiled in a blog post where their first product was described as "a plant-based smoked salmon made from carrots, uses simple ingredients including locally-sourced bull kelp and Omega-3s from flax oil to create a smoky, savoury, delicious plant-based alternative."

Consistent with our survey results, public statements from alternative seafood companies more often reflect a focus on nutritional equivalence rather than enhancement. However, a representative of <u>BlueNalu</u> was quoted in a 2022 article as stating "We can actually create cells, if we want, that have exactly the same profile [as wild-caught fish], or we can create cells that, for example, might have 90 percent omega-3 [fatty acids]."

*as of March 2024

How are omega-3s added to cultivated products?

For companies focusing on cultivated products (including hybrids), we asked what strategies they use to introduce EPA, DHA, and other omega-3s into their products. Companies reported using a variety of strategies (n=9 for both questions, Figure 19), with addition to the culture media being the most common (corresponding to Option 3a in Figure 20).

Company respondents also reported using other strategies. Post-harvest addition (Option 1 in Figure 20) was used by three respondents for other omega-3s and two for EPA/DHA. Incorporation into the scaffold material (Option 2 in Figure 20) was used by two respondents for other omega-3s and one for EPA/DHA. Production by co-cultured animal cells (corresponding approximately to Option 3b in Figure 20) was used by one respondent for both, while production by the cultivated cells themselves (corresponding approximately to Option 4 in Figure 20) was used by one for other omega-3s and two for EPA/DHA. The companies using these last two strategies either reported that their answers were based on targets rather than fully characterized prototypes or skipped that question. Responses to open-ended questions also indicated that companies pursuing these strategies are currently at a fairly exploratory stage, for example:

Q19. Please elaborate on the extent and nature of your challenges when sourcing omega-3-containing ingredients.

"The cells have some capacity to generate them themselves. In the future we will investigate how to encourage the cells to produce more omega's themselves."

Finally, some respondents reported not doing anything to introduce other omega-3s (two responses) or EPA/DHA (three responses) into their products.

Methods used to introduce omega-3s into cultivated meat and seafood prototypes



Figure 19. Methods currently in use to introduce non-EPA/DHA omega-3s (A) and EPA/DHA (B) into cultivated product prototypes. Q40. How does your company currently introduce omega-3s (besides EPA and DHA) into your cultivated products or prototypes? (Please select all that apply.) Q37. How does your company currently introduce EPA and DHA into your cultivated products or prototypes? (Please select all that apply.) Colors represent product/prototype status (Q33).

Cultivated meat and seafood

For the most part, the need for omega-3 ingredients is agnostic to the method used for the production of alternative protein products. However, there are some additional considerations in the use of omega-3 ingredients in cultivated meat. Briefly, the cultivated meat production process consists of 1) identifying an appropriate starting cell population, 2) growing and then differentiating the cells in appropriate culture media, often with the help of a three-dimensional scaffold, and 3) harvesting the resulting tissue and performing any final processing steps needed for the intended end product. For a more detailed discussion of the production process, please see <u>The Science of Cultivated Meat</u>.

The introduction of omega-3 ingredients can, in theory, happen <u>at any point in this process</u>

(Figure 20), including by cultivating cells that can produce their own EPA and DHA from simpler inputs. This could be accomplished by engineering the cultivated cells themselves (Zhu et al. 2014) or by relying on the tendency of certain cell types, such as freshwater fish hepatocytes (liver cells), to convert ALA into EPA and DHA (Tocher 2003; Suito et al. 2018). The mechanisms of fatty acid transport across the cell membrane are not entirely understood but are thought to involve membraneassociated transporters including fatty acid binding protein (FABP), several fatty acid transporter proteins (FATPs), the fatty acid translocase CD36, and caveolin-1 (Glatz, Luiken, and Bonen 2010). Whatever the mechanism, it has been shown that cultured tuna cells readily take up EPA, DHA, and other fatty acids from the culture medium (Scholefield and Schuller 2014).



Figure 20. Possible methods for incorporating EPA and DHA into cultivated meat or seafood products. According to our results, addition of EPA and DHA to the culture media (corresponding to Option 3a) is the most common method. Created with Biorender.com.

While this has not been confirmed experimentally, it would be reasonable to hypothesize that the introduction of EPA and DHA later in the production process (e.g., during a final processing step) might result in a product where EPA and DHA are mostly present extracellularly, whereas introducing them early in the process or through cell line engineering might cause them to end up mostly intracellular (bottom of Figure 20). Whether and how this might impact flavor, texture, mouthfeel, or shelf life would need to be determined empirically for a given product.

Future targets and anticipated needs

While algae remains the top source of interest, companies and researchers are interested in diversifying their EPA and DHA sources to include other marine microorganisms and recombinant sources. Interest in different chemical forms of EPA and DHA mostly mirrors current usage, with free fatty acids and triglycerides ranked highest, followed by phospholipids. Few respondents currently use encapsulated ingredients, but around half are interested in doing so. We did not see a clear preference between more- or less-refined forms of omega-3 ingredients.

When asked about their targets for five years in the future, most companies indicated that they plan to target products with equivalent total omega-3 and EPA/DHA content to conventional meat or seafood. Twice as many respondents planned to target higher content compared to today, although these were still a small proportion. Companies focusing on terrestrial meat also expressed some interest in pursuing equivalent or enhanced total omega-3 and EPA/DHA content as a future goal. Consistent with their anticipated increase in focus on omega-3s in their future products, companies predicted needing to source substantially more omega-3 ingredients and facing increased challenges in doing so.

What ingredients are companies and researchers interested in?

We asked both academic and company respondents several questions about the types of EPA and DHA ingredients they were interested in using in the future. Algae was the most commonly selected source, but respondents were also interested in alternatives, especially native expression by other marine microorganisms and recombinant expression in either microorganisms or plants, with somewhat less interest in cell-free sources (n=21, Figure 21). The one respondent who selected "Other" was interested in cultivated fish fat cells as an EPA/ DHA source. Responses to this question contrasted somewhat with those to the previously mentioned question about current sources of EPA and DHA, where algae was by far the most common response (n=21). This indicates that respondents would be interested in diversifying their ingredient sources.





Figure 21. Comparison of EPA/DHA ingredient sources currently in use versus those that respondents expressed interest in using. Q22. Where are your EPA and DHA sources coming from? (Please select all that apply.) Q24. What additional EPA and DHA sources or production mechanisms are of most interest to your company or lab?

Alternative omega-3 sources

The quest for sustainable and diverse sources of essential omega-3 fatty acids, particularly EPA and DHA, has gained significant attention as an alternative to fish-derived oils. While fish have long been the primary source of EPA and DHA, concerns about <u>wild fish populations</u> and <u>supply gaps</u> have spurred research into other potential sources. These include natural and engineered oleaginous (lipid-rich) microorganisms, engineered oil-seed crops, and cell-free production systems (Figure 22). These same production systems might also serve as sources of other valuable compounds (e.g., vitamins D and K from microalgae (Del Mondo et al. 2020)) for use in alternative proteins or other sectors, thereby contributing to a more circular bioeconomy. The benefits of such compounds could also be advantageous when using less-processed omega-3 ingredients (see Figure 26).



Figure 22. Some possible sources of EPA and DHA that could represent inputs for cultivated meat and seafood production. Non-recombinant sources (top) rely on microorganisms that naturally produce EPA and DHA, while recombinant sources (middle) are those where the relevant gene has been introduced into an organism that is ideally easy to grow and harvest and accumulates a large percentage of lipids. Cell-free and in vitro enzymatic systems (bottom) could be promising, but are currently at an earlier stage of technological development. Created with Biorender.com.

Microalgae & other microorganisms

Unlike animals, who cannot produce EPA and DHA on their own and rely on dietary intake, many microorganisms naturally express the necessary enzymes for EPA and DHA synthesis. Many oleaginous microorganisms can accumulate substantial amounts of lipids in their cells. They can serve as natural or recombinant sources of <u>polyunsaturated fatty acids</u> (PUFAs), including omega-3 EPA and DHA, or omega-6 <u>arachidonic acid</u> (ARA). Non-recombinant microbial sources come from naturally occurring strains of bacteria, microalgae, fungi, and yeasts without genetic modification. Recombinant production involves genetic engineering techniques to enhance the production of EPA and/or DHA in many of these same microorganisms, allowing for greater control and optimization of lipid synthesis pathways. Microalgae are the primary producers of EPA and DHA in the natural environment, thus they have received the most commercial interest. This diverse group can be divided into photoautotrophic microalgae and heterotrophic microalgae-like marine protists.

Heterotrophic microalgae

Heterotrophic microalgae, meaning those that obtain carbon from organic compounds like glucose rather than CO₂, are commercially important sources of DHA. Among these, thraustochytrid and dinoflagellate algae are the primary source of DHA-rich algal oils. While technically algae, they are marine protists that are non-photosynthetic and obligate heterotrophs. These microorganisms can be cultivated in controlled, industrial bioreactors where conditions like temperature, pH, and nutrient availability are optimized to enhance DHA production. Notably, species such as Schizochytrium, Aurantiochytrium, Crypthecodinium, and Ulkenia can accumulate lipids over 50 percent cell dry weight and are known for their ability to efficiently produce DHA-rich oils at 30-60 percent of the total lipid content (Table 1). This makes them excellent DHA sources for human consumption. However, most do not produce high concentrations of EPA (Martins et al. 2013; Saini et al. 2021). For instance, Crypthecodinium produces high DHA but very low EPA, which makes it suitable for infant formula (GRN 0041) (CFSAN 2023). On the other hand, some Schizochytrium strains are naturally capable or have undergone laboratory-driven evolution to produce increased concentrations of EPA (Saini et al. 2021; EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) 2014)).

Photoautotrophic microalgae

Photoautotrophic microalgae are another source of omega-3 fatty acids and tend to produce higher levels of EPA than heterotrophic microalgae. These microalgae utilize inorganic CO₂ for growth and light energy for photosynthesis. However, many phototrophic microalgae can be grown autotrophically and/or heterotrophically with sugar, known as mixotrophy. These microalgae can be grown outdoors in open-raceway ponds or enclosed photobioreactors for increased process control, though there are productivity and capital investment tradeoffs. While many phototrophic microalgae produce PUFAs, only a few commercially relevant strains naturally produce high levels of EPA and/or DHA, such as Nannochloropsis sp., Phaeodactylum tricornutum, and Odontella aurita (Martins et al. 2013; X.-N. Ma et al. 2016) (Table 1).

Beyond microalgae, oleaginous bacteria and fungi can also accumulate over 20 percent of lipids in their cells. They can be cultivated under heterotrophic conditions in controlled industrial bioreactors with higher productivity, shorter batch lengths, highdensity fermentation, and feedstock flexibility (Qin et al. 2023).

Oleaginous fungi

Commercially relevant fungal strains with high PUFA production include the yeast *Yarrowia lipolytica* and fungi *Mortierella alpina*. However, they do not natively produce high concentrations of EPA or DHA. In wild-type *Y. lipolytica*, omega-6 linoleic acid is the major PUFA produced. Wild-type filamentous fungi *Mortierella alpina* can produce high concentrations of omega-6 ARA. *M. alpina* can also produce high concentrations of EPA, but only at low temperatures, greatly impacting commercial techno-economics (Okuda et al. 2015). The greatest opportunity for these oleaginous fungal strains to produce high concentrations of EPA and DHA is through recombinant engineering.

Oleaginous bacteria

Compared to microalgae and fungi, bacteria are not usually considered high producers of essential fatty acids. Deepsea marine bacteria, such as Moritella and Shewanella, are known for their high PUFA production, which is thought to enable their growth in cold environments as their PUFA production is connected to low temperatures (2-4 °C) and high pressure (Kannan, Rao, and Nair 2021; Yoshida et al. 2016). These strains provide crucial understanding and pathway discovery for bacterial PUFA production but have yet to be commercially relevant for production due to their lower lipid content and dependence on cold temperatures for PUFA production. Other oleaginous bacteria like *Rhodococcus* can produce very high levels of lipids (>70% cell dry weight). While they are not typically known for naturally producing high levels of PUFAs, there are opportunities for recombinant engineering (Alvarez et al. 2021).

Recombinant microorganisms

For many oleaginous microorganisms, recombinant strain engineering advances enable heterologous (non-native) expression of omega-3 synthesis pathways that can significantly improve EPA and/ or DHA productivity. The omega-3 pathway has a variety of desaturases and elongases with varying activity and specificity (Cao et al. 2022). Thus, there have been many different metabolic engineering approaches in a wide range of microorganisms. For microalgae, there has been a flurry of strain engineering strategies for Aurantiochytrium sp., Schizochytrium sp., P. tricornutum, Nannochloropsis sp., and Dunaliella sp. that yielded improved DHA and/or EPA concentrations (Jesionowska et al. 2023; Shi et al. 2018; Jakhwal et al. 2022; W. Ma et al. 2022). However, commercially, there are no

recombinant sources of algal DHA or EPA oil on the market to our knowledge. While DSM-Fermenich has developed recombinant microalgal strains (*Schizochytrium sp*) to increase EPA production and a higher EPA:DHA ratio, they also appeared to have achieved comparable results with non-recombinant strain mutagenesis, adaptive evolution, and process improvement (Bayne and Zirkle 2019; Pfeifer et al. 2013). Therefore, it is unclear if recombinant technology is needed in the case of microalgal species that already express the necessary enzymes for EPA and DHA synthesis.

Significant engineering efforts have also been made in oleaginous fungi. For instance, M. alpina has been engineered for overproduction of >20 percent EPA of the total fatty acids (Okuda et al. 2015; 陈永泉 et al. 2014). The dramatically increased EPA production at higher temperatures can greatly improve production costs for *M. alpina*. Similar metabolic engineering strategies, along with process optimization, have been implemented with Y. lipolytica, which does not natively produce EPA. These efforts increased EPA production to up to 25 percent dry biomass (Xie et al. 2017). Commercially, a recombinant Y. lipolytica strain was engineered by DuPont to produce around 35 percent (w/w) lipids and 15 percent (w/w) EPA. These EPA-rich lipids were commercially produced by CPKelco and sold in the USA as NewHarvest™ EPA oil for human consumption after being designated Generally Recognized as Safe (GRAS, GRN 0355) by the U.S. Food and Drug Administration (FDA) in 2011 (Abeln and Chuck 2021; CFSAN 2023).

To our knowledge, there are no current recombinant sources of fungal EPA- or DHA-rich oil on the market. A shifting consumer perception, increased demand, and improvements in production costs could enable the commercialization of recombinant omega-3 oil sources.

Organism	Lipid content (% cell DW)	EPA (% TFA)	DHA (% TFA)	Reference
Phototrophic micro	oalgae			
Chlorella sp.	28-53	•	•	(Udayan et al. 2022; Couto et al. 2022)
Dunaliella sp.	5-50 28-33*	0-2 23-28*	0-15 •	(XN. Ma et al. 2016; Hosseinzadeh Gharajeh et al. 2020) (Shi et al. 2018)*
Phaeodactylum tricornutum	18-57	22-57	<2	(Martins et al. 2013; Saini et al. 2021)
Odontella aurita	10-20	9-25	•	(An et al. 2023)
Isochrysis galbana	13-30	3-4	12	(Liu et al. 2022; Señoráns, Castejón, and Señoráns 2020)
Pavlova sp.	12-30	22	•	(Udayan et al. 2022)
Nannochloropsis sp.	15-60	5-45	1-3	(XN. Ma et al. 2016; Hulatt et al. 2017)
Heterotrophic mic	roalgal marine proti	sts & dinoflagella	ates	
Aurantiochytrium sp.	30-65	<1	15-40	(Liu et al. 2022; Hien et al. 2022)
Schizochytrium sp.	40-67	0-2 10*	~30-60 45*	(Martins et al. 2013; Liu et al. 2022) (W. Ma et al. 2022)*
Schizochytrium sp. (DSM)	40-67	10-20	30-40	(EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) 2014; Martins et al. 2013)
Thraustochytrium sp.	~40	1-8	38-49	(Martins et al. 2013; Kaliyamoorthy et al. 2023)
Ulkenia sp.	20-52	•	15-30	(Saini et al. 2021)
Crypthecodinium sp.	0-0	•	35-65	(Liu et al. 2022; Saini et al. 2021)

Table 1. Lipid content of exemplar non-recombinant and recombinant* microorganisms.**

Oleaginous yeast & fungi

Yarrowia lipolytica	20-50	• 50*	•	(Abeln and Chuck 2021; Carsanba et al. 2020) (Xie, Jackson, and Zhu 2015)*
Mortierella alpina	20-50	0-6 26-31*	0-4	(Qin et al. 2023; Vadivelan and Venkateswaran 2014) (Okuda et al. 2015; Tang et al. 2018)*

*Recombinant microorganism examples

**Non-exhaustive list of oleaginous microorganisms. This offers a snapshot of the most common, commercially relevant strains. DW = dry weight; %TFA = total fatty acid content. Recombinant strain examples are provided for selected species, with asterisks indicating comparisons to fatty acids in the wild-type genus or species.



Microbial commercial landscape

Commercially, several PUFA-rich microbial products have regulatory clearance for use in food via the FDA GRAS process and Novel Foods status through the European Food Safety Authority (EFSA) novel foods notification processes. These PUFA-rich oils are also produced for dietary supplements that often undergo separate regulatory clearance, such as the FDA New Dietary Ingredient Notification (NDIN) process.

Omega-3 DHA-rich algal oils produced from the heterotrophic microalgae Aurantichytrium limacinum, Crypthecodinium cohnii, Schizochytrium sp., and Ulkenia sp. have been designated GRAS for several companies, and have received Novel Foods status through EFSA. These include over 20 DHA-rich algal oils with Novel Foods and GRAS designations for companies including ATK Biotech, BASF, CABIO Biotech, DSM-Fermenich, Fermentalg, Hubei Fuxing Biotech, Lonza, Mara Renewables, and Runke Biotech (European Commission for Novel Foods 2024; CFSAN 2023). There are also many DHA-rich algal oils with NDIN dietary supplement designation from companies such as Algarithm Ingredients, DSM, Heliae, OmegaTech, and Solarvest (CFSAN NDI 2024).

Omega-3 EPA-rich algal or fungal oils are commercially limited as food ingredients despite their commercial application as dietary supplements, aquaculture feed, and pet food. The only EPA-rich oil designated as GRAS is from the recombinant *Y. lipolytica* engineered by DuPont (<u>GRN 0355</u>).

However, this does not appear to be commercially active. DSM-Fermenich has received GRAS (GRN 0137) and Novel Foods (EU 2022/1365) designation for their non-GMO DHA- and EPA-rich algal oil produced from Schizochytrium sp. Elsewhere, EPArich oil (27%) from the microalgae P. tricornutum produced by Simris had a favorable opinion as a novel food by the Food Safety Authority of Ireland (FSAI). However, its review under EFSA has yet to be granted (EFSA NDA 2023). And, while the biomass of the EPA-rich microalgae Odontella aurita has "Qualified Presumption of Safety" (QPS) status with EFSA, any extraction or refinement of an EPA-rich oil would require evaluation and designation. Even though food ingredient use is limited, there are approved EPA-rich dietary supplements, such as Nannochloropsis EPA-rich oils produced by Qualitas Health in NDIN 826 (CFSAN NDI 2024).

Omega-6 ARA-rich fungal oils produced from *M. alpina* have also received GRAS and Novel Foods designations as food/beverage ingredients for several companies. Examples include BASF (<u>GRN 0963</u>), Hubei Fuxing BIotech (<u>GRN 1115</u>), Linyi Youkang Biology Co. (<u>GRN 0730</u>), and Suntory Ltd (<u>2008/968/EC</u>). Further, ARA-rich oil from the fungus *Mortierella alpina* has been listed as a novel food (<u>EU 2017/2470</u>) under certain uses (European Commission for Novel Foods 2024). The commercial application of *M. alpina* for ARA could enable future designation for recombinant EPA-rich oil producers of *M. alpina*, given the limited commercial microbial sources.

Production of microbial Omega-3s

Production processes for any phototrophic microalgae, heterotrophic microalgae, and oleaginous fungi are highly comparable, especially in downstream processing. Industrial production of microbial omega-3 consists of four primary steps: (1) growth under controlled conditions, (2) harvest recovery of the lipids and biomass, (3) lipid extraction, typically solvent-based, from the wet or dried biomass, and (4) lipid purification to obtain a refined oil. Growth will vary by microorganism whether it is produced in open-ponds phototrophically, in closed photobioreactors photoor mixotrophically, or in closed bioreactor tanks via heterotrophic growth.

Commercially, microbial omega-3 sources are becoming more cost-competitive due to the flattening fish oil supply and projected market growth for omega-3 supply. Fish EPA/DHA-rich oil extract prices historically ranged from \$300–800/ tonne until around 2005. However, increased demand and limited supply have recently raised the price range from \$1500–2500/tonne fish oil (Oliver et al. 2020; dos Reis et al. 2024; Chauton et al. 2015). A standard fish oil at \$2400/tonne with 30 percent EPA/DHA would have a normalized price of \$8/kg EPA and DHA (Chauton et al. 2015). However, prices are likely higher for further refined omega-3rich fish oil extracts with higher quality and concentration.

These price increases have made microbial production more cost-competitive. Recent technoeconomic models for heterotrophic microalgae production have estimated production costs from \$16 to 40 per kg of omega-3-rich oil (Russo et al. 2022; Flevaris et al. 2021). Estimates for phototrophic omega-3-rich microalgae production have ranged from \$12 to 100 (Wan Razali and Pandhal 2023; Chauton et al. 2015; Schade and Meier 2021). These microbial oils offer several benefits over fish oil, such as increased sustainability, reduced pressure on wild fish populations such as anchovy and menhaden, and safer/higher quality oils free from bioaccumulated toxic substances like dioxins and heavy metals. These benefits may afford microbial PUFA oils their initial higher price entry for certain products, such as infant formula and nutraceuticals (Mamani et al. 2019; Davis et al. 2021; Bartek et al. 2021).

Plant molecular farming

Plant oils produce shorter-chain omega-3 fatty acids, such as ALA, and lack native metabolic pathways that synthesize longer-chain omega-3s like EPA and DHA. However, crops can be modified to integrate genes from microorganisms that produce EPA and DHA, allowing these crops to create long-chain omega-3 PUFAs (Qin et al. 2023). This transgenic process of using crops to produce non-native, desirable compounds is referred to as plant molecular farming (PMF).

Implementing PMF to produce long-chain omega-3 PUFAs has been an active area of research for decades, with the production of EPA and DHA demonstrated in Arabidopsis (Qi et al. 2004), Camelina sativa (Ruiz-Lopez et al. 2014; Usher et al. 2015, 2017), Brassica napus (canola) (Petrie et al. 2020; MacIntosh et al. 2021), and other oilseeds (Kinney et al. 2004) (Table 2). Two PMF pathways have been explored: (1) The anaerobic pathway, consisting of polyketide synthase (PKS)-like PUFA synthase biosynthesis, and (2) The aerobic pathway, consisting of the alternative desaturase and elongase pathway. The aerobic pathway is by far the more common and successful approach to modify oilseeds, although preliminary results from Dow and DSM demonstrate that transgenic canola with a microalgal PKS-like pathway was able to produce modest levels of DHA (up to 3.7% of seed oil) and EPA (up to 0.7%) (Walsh et al. 2016).

Generally, producing omega-3 fatty acids using PMF has focused on EPA and DHA synthesis in C. sativa or canola by overexpressing desaturase and elongase genes for multiple metabolic steps that transform shorter fatty acids such as oleic acid, linoleic acid, and ALA. Yield10 Bioscience and Rothamsted Research have developed an EPA-rich C. sativa and recently contracted fifty acres in Chile to produce their EPA C. sativa cultivar at scale. The group has worked on developing C. sativa cultivars with high DHA content as well (Ruiz-Lopez et al. 2014). Further, the team has demonstrated that, in human subjects, supplemented EPA and DHA produced by C. sativa incorporated into blood lipids and accumulated in plasma lipids as well as EPA and DHA from fish oil (West et al. 2021).

CSIRO, GRDC, and Nuseed (as a team), as well as BASF and Cargill (as a team), are leading efforts to develop canola cultivars with seven to eight yeast or algae genes to enable the production of DHA and EPA (Napier, Olsen, and Tocher 2019). The DHA canola produced by the Nuseed team has been through regulatory approval in <u>Canada</u>, and U.S. FDA recognized their commercial formulation of DHA-rich canola oil as a <u>New Dietary Ingredient</u> allowing use as a nutraceutical supplement in the U.S.

Additionally, for PMF omega-3 fatty acid ingredients to become commercially feasible, they must be produced efficiently at scale. As a result, current research focus areas include increasing the percentage of EPA and DHA produced in transgenic oilseeds. That being said, it has been estimated that 2.5 million hectares (ha) of canola (about 6% of <u>current global canola ha</u>, roughly the size of the state of Wyoming) containing 10–15% DHA would provide the DHA yield of all fish oil harvested globally (Zhou et al. 2019).

The economics of the commercial production of PUFAs in transgenic oilseeds is not well-established, but plants are generally considered low-cost, high-speed platforms for recombinant protein production. Infrastructure investment in commercial crop production is already well-established and downstream processing costs of plants can be comparable to those of microbial and mammalian cells (Feng et al. 2022). Moreover, plants can carry out many post-translational modifications for the production of complex proteins and do not produce endotoxins, making them more suitable for certain applications than other expression platforms. When producing ingredients like long-chain omega-3 fatty acids via transgenes, whether a mammalian, microbial, or plant platform is most economical will highly depend on the target fatty acid, host identity, production pathway optimization, and downstream processing requirements and should be evaluated through techno-economic analyses on a case-bycase basis.



Сгор	Cultivar	Seed lipid content*	EPA (% TFA)	DHA (% TFA)	Reference
Canola	LBFLFK (BASF)	40-50%	7%	1%	(Napier, Olsen, and Tocher 2019)
Canola	NS-B50027-4 (Nuseed)	40-50%	<0.5%	10%	(MacIntosh et al. 2021)
C. sativa	RRes_EPA (Rothamsted)	35-42%	24%	0%	(Ruiz-Lopez et al. 2014)
C. sativa	RRes_DHA (Rothamsted)	35-42%	11%	8%	(Ruiz-Lopez et al. 2014)

Table 2. EPA and DHA concentrations in various oilseeds.

*Estimated based on wildtype canola and camelina seed oil content (Zum Felde et al. 2007; University of Wisconsin-Madison, n.d.)



Figure 23. DHA, EPA, and ALA content as a percentage of total fatty acids (FA) for salmon, *Camelina* (wild-type and modified), and four algal species (including recombinant strains for two). For values with a range, the number shown here is the midpoint. Sources: (Molversmyr et al. 2022; Ruiz-Lopez et al. 2014; Yuan and Li 2020; Martins et al. 2013; Saini et al. 2021; Kaliyamoorthy et al. 2023; Liu et al. 2022; W. Ma et al. 2022; Abeln and Chuck 2021; Carsanba et al. 2020; Xie, Jackson, and Zhu 2015).

Cell-free or in vitro enzymatic systems

Cell-free systems contain all the necessary transcriptional and translational machinery of a cell without the cell membrane-usually in the form of a cell lysate-and can be induced to produce various proteins simply by adding the corresponding DNA sequence(s) (Tinafar, Jaenes, and Pardee 2019). If the sequences added encode one or more enzymes, it is also possible to produce various small molecules (Pardee et al. 2016; Rasor et al. 2021). Rather than relying on a cell-free system for transcription and translation, it is also possible to reconstitute biosynthetic pathways using purified enzymes (Yu et al. 2011). Those enzymes can ultimately come from any source, including those described above. While the terminology used in the literature varies somewhat, for our purposes, we will use "cellfree systems" to mean those systems where DNA is used as an input (i.e., a "programmable liquid" as described by Tinafar et al. (2019)) and "in vitro enzymatic systems" to refer to those where purified enzymes are the input.

As a newer technology, the techno-economics of cell-free production is not yet well understood. An assessment of monoclonal antibody production from Chinese hamster ovary (CHO) cell extracts estimated that cell-free production would be around twice as expensive as traditional cell-based methods at small scales and that this gap could grow at larger scales (Thaore et al. 2020). A separate study estimated that producing antibody-drug conjugates from cell-free E. coli lysates would cost 80 percent more than producing the same products using CHO cells (Stamatis and Farid 2021). However, CHO cells have received a great deal of investment over the years, while the same is not true of cell-free systems (Melinek et al. 2022). Thus, current techno-economic assessments may not similarly reflect the achievable potential of both technologies.

While we are unaware of any life cycle assessments of cell-free systems, such systems present theoretical opportunities such as increased ease of using side streams or one-carbon feedstocks that could offer sustainability advantages (Rasor et al. 2021). It is also worth noting that lysate production was identified as a major cost driver in cell-free production (Stamatis and Farid 2021). In theory, this could suggest a potential advantage for systems relying on in vitro enzymatic methods. This strategy could take advantage of certain features of cell-free systems, such as the lack of impacts of the product on cell viability (Rasor et al. 2021), while circumventing resource-intensive steps such as cell lysate production.

Currently, the costs and environmental impacts of cell-free or in vitro enzymatic EPA and DHA production are difficult to predict. The available evidence makes it difficult to either recommend or rule out cell-free and in vitro enzymatic systems as a source of affordable and sustainable long-chain omega-3 ingredients. However, there is evidence that cell-free systems may be more favorable from a cost perspective when it comes to applications such as personalized or orphan medicine due to their easy adaptability to a wide variety of proteins (Melinek et al. 2022; Thaore et al. 2020), providing a clear incentive for their further development. As the technology evolves, it may become clearer whether there is value in exploring these methods for omega-3 ingredient production.

Interest in omega-3s in different forms (n=18, Figure 24) roughly mirrors current usage (n=15). Triglycerides, free fatty acids, and phospholipids are both the most often used and the most frequently listed as ingredients of interest.

Several of the open-ended answers (Q32) reflected an openness to a variety of different ingredient types as long as they met certain criteria. For example:

Q32. Please share any further details related to omega-3 (including EPA and DHA) ingredient volumes and current challenges that aren't captured by your answers above. If you'd like to provide any clarifications related to your answers above, please do so here.

"We would be excited to work with different formulations, encapsulation methodologies, particularly if there is strong potential for scaleability from the start."

Another respondent mentioned that the chemical form they were most interested in was whatever was most temperature stable.

Forms of EPA and DHA currently in use versus forms of interest



Figure 24. Comparison of the chemical forms of EPA/DHA ingredients currently in use versus those that respondents expressed interest in using. Q26. In what form(s) are you currently sourcing EPA and DHA? (Please select all that apply.) Q28. In what form(s) are you most interested in sourcing EPA and DHA? (Please select all that apply).



Only three respondents reported that they currently use encapsulated ingredients, but many expressed interest in doing so (n=25, Figure 25). This suggests a potential white space for new ingredients that meet the needs of alternative meat and seafood producers. It is also noteworthy that this result aligns with the responses to the question about specific challenges associated with omega-3 ingredients, where two of the top five responses (shelf life and off-flavors) have a clear association with oxidation processes. Oxidation, shelf life, and stability were also mentioned frequently in responses to open-ended questions about knowledge gaps and obstacles to omega-3 fortification (discussed on page 51-52).

Do respondents use encapsulated omega-3 ingredients?



Figure 25. Q30. Do you use encapsulated omega-3 ingredients? Colors represent company/academic status (Q7) and company stage (Q11)

Encapsulation and oxidation

Omega-3 fatty acids contain multiple double bonds that are prone to oxidation and rancidity when exposed to air, heat, light, chemical and enzymatic oxidizers, and transition metals like iron and copper. The most common causes of unsaturated lipid oxidation are longer storage duration and high storage or cooking temperatures. Oxidation of omega-3 fatty acids negates their nutritional benefits and produces undesirable odors and flavors (Sardenne et al. 2021). Solutions that <u>reduce</u> <u>omega-3 oxidation and rancidity</u> are desirable for the alternative seafood industry to create nutritious and delectable products. Of these solutions, encapsulation of omega-3 fatty acids to reduce double-bond oxygen exposure has demonstrated promise (Drusch and Mannino 2009).

Survey respondents indicated that two of the top five specific challenges associated with omega-3 ingredients were shelf life and off-flavors (Figure 8), both of which are negatively affected by lipid oxidation processes. Still, only three respondents reported that they currently use encapsulated ingredients, but many expressed interest in doing so (Figure 25). This suggests a potential white space opportunity for new and existing businesses to create oxidatively stable omega-3 ingredients that meet the needs of alternative meat and seafood producers.

Respondents have different needs when it comes to the level of refinement desired, but all three products (concentrates, refined oils, and minimally processed oils) are considered acceptable by a substantial proportion of companies and researchers (n=21, Figure 26). According to one respondent who selected all three options, there are competing priorities that favor higher or lower levels of refinement. More concentrated ingredients make it possible to introduce a large amount of omega-3 fatty acids without diluting the product, while less processed, more "natural" ingredients may have advantages from a marketing perspective. These less processed ingredients may also come with additional benefits in the form of vitamins and minerals present in the source organism. Such tradeoffs may underlie the lack of a clear overall preference for more or less refined ingredients.

Types of omega-3 ingredients compatible with respondents' needs



Figure 26. Q31. What type(s) of omega-3 ingredients are compatible with your needs? (Please select all that apply. Colors represent company/ academic status (Q7) and company stage (Q11).

Where do companies hope or expect to be in 5 years when it comes to omega-3s?

Respondents representing alternative meat or seafood companies were surveyed on their anticipated products in five years. They were asked to assume an "optimistic but realistic rate of growth" for their company. Such projections have a great deal of inherent uncertainty and should be interpreted as "best guesses" rather than firm predictions. All results presented in this section reflect answers from company respondents only.

Omega-3 and EPA/DHA targets for future products

In sharp contrast to the answers to the earlier question about the content of current products (n=22 for total-omega3 and n=23 for EPA/DHA, including only respondents from companies), most companies plan to target equivalent total omega-3 and EPA/DHA content (n=22 for both, Figure 27) in future products. While those interested in higher content are still in the minority, twice as many respondents indicated this as a future target compared to those who were currently pursuing such strategies. We saw a similar but smaller shift toward higher omega-3 and EPA/DHA targets in the future scenario in survey 2 (n=17 for current total omega-3 content, n=16 for all others, Figure 28). In both surveys, we saw only minor differences in responses to the questions about EPA/DHA and total omega-3 future targets.





Figure 27. Comparison of the total omega-3 (A) and EPA/DHA (B) content of current products/prototypes versus five-year targets. A: Q35. Compared to conventional equivalents (e.g., conventional salmon if your company makes plant-based salmon), what is the total omega-3 content of your company's current products or prototypes? Q47. Compared to conventional equivalents (e.g., conventional salmon if your company makes plant-based salmon), what is your target for the total omega-3 content of your company's future products within the next 5 years? B: Q36. Compared to conventional equivalents (e.g., conventional salmon if your company makes plant-based salmon), what is the EPA/DHA omega-3 content of your company's current products or prototypes? Q48. Compared to conventional equivalents (e.g., conventional salmon if your company makes plant-based salmon), what is your target for the EPA/DHA omega-3 content of your company's future products within the next 5 years?

Total omega-3 and EPA/DHA content (compared to conventional meat or seafood) of current products or prototypes versus five-year targets (survey 2)



Figure 28. Comparison of the total omega-3 (A) and EPA/DHA (B) content of current products/prototypes versus five-year targets in survey 2. A: Q2-216. Compared to conventional equivalents (e.g., conventional salmon if your company makes plant-based salmon), what is the total omega-3 content of your company's current products or prototypes? If your company produces multiple products and your answer differs depending on the product, please use the text box at the end of this section to provide further details. Q2-217. Compared to conventional equivalents, what is the EPA/DHA omega-3 content of your company's current products or prototypes? B: Q2-219. Compared to conventional equivalents, what is your target for the total omega-3 content of your company's future products within the next 5 years? Q2-220. Compared to conventional equivalents, what is your target for the EPA/DHA omega-3 content of your company's future products within the next 5 years?

Omega-3s in alternative meat versus seafood

The shift toward greater interest in omega-3 equivalence or enhancement included companies focused on terrestrial meat (Figure 29). No survey 1 respondents with a focus on terrestrial alternative meat products (whether exclusively or otherwise) indicated that they were currently producing or targeting omega-3-equivalent or enhanced products. However, the majority of those who responded to the questions about future targets indicated that they plan to target at least equivalent content, and some plan to target higher content (Figure 29). A similar but smaller shift was apparent among alternative terrestrial meat-focused companies in survey 2 (Figure 30).





Figure 29. Current (A) and five-year (B) targets for EPA/DHA content, colored according to the company's focus on seafood, terrestrial meat, or both. Results were similar for total omega-3 content. A: Q36. Compared to conventional equivalents (e.g., conventional salmon if your company makes plant-based salmon), what is the EPA/DHA omega-3 content of your company's current products or prototypes? B: Q48. Compared to conventional equivalents (e.g., conventional salmon if your company makes plant-based salmon), what is your target for the EPA/DHA omega-3 content of your company's future products within the next 5 years? Colors represent species focus (Q9-10, manually aggregated).

Total EPA/DHA content of current products or prototypes versus five-year targets in relation to focus on terrestrial meat or seafood (survey 2)



Figure 30. Current (A) and five-year (B) targets for EPA/DHA content in Survey 2, colored according to the company's focus on seafood, terrestrial meat, both, or neither. Results were similar for total omega-3 content. A: Q2-217. Compared to conventional equivalents, what is the EPA/DHA omega-3 content of your company's current products or prototypes? B: Q2-220. Compared to conventional equivalents, what is your target for the EPA/DHA omega-3 content of your company's future products within the next 5 years? Colors represent species focus (Q2-6). Respondents focused on neither meat nor seafood were a fairly diverse group that included companies focused on ingredients and inputs, oils and fats, and dairy, with many respondents selecting multiple options.



We also asked survey 2 participants a more general question about their company's level of interest in omega-3-enhanced products. Responses to this question depended strongly on the company's product focus (Figure 31). The two exclusively seafood-focused respondents were either developing or actively considering omega-3-enhanced products. Among those focused on terrestrial meat (with or without seafood), the most common response was that enhanced products were not a current priority but could be a possibility depending on consumer demand. Among those with neither focus, the most popular response was that enhanced products were under active consideration. This result is difficult to interpret with confidence due to the diversity of the companies, but may signal a substantial level of interest among ingredient suppliers.

Overall, it appears that for most alternative terrestrial meat companies, achieving equivalent or enhanced omega-3 content—whether total or EPA/ DHA—is not a current focus. Equivalent or enhanced content in future products is a priority for some but not others. This ambivalence may reflect a lack of data on what consumers want. For seafood-focused companies, on the other hand, equivalent content is a current priority for many, and a future priority for most. A few seafood-focused companies reported that omega-3-enhanced products were a current or future priority.

Approach to "omega-3-enhanced" products (survey 2)



Figure 31. Q2-215. Producing alternative meat, seafood, eggs, and dairy products with higher levels of omega-3 fatty acids (e.g., long-chain omega-3s like EPA and DHA) compared to their conventional counterparts is a possible benefit of alternative protein products. Which of the following most accurately describes your company's approach to such "omega-3-enhanced" products? Colors represent species focus (Q2-6). Respondents focused on neither meat nor seafood were a fairly diverse group that included companies focused on ingredients and inputs, oils and fats, and dairy, with many respondents selecting multiple options.

Anticipated ingredient volumes

Companies also reported expecting to purchase larger volumes of omega-3 ingredients in the future (n=18) compared to today (n=22) (Figure 32). For simplicity, participants were asked to assume that the omega-3 and EPA/DHA concentrations of their future ingredients would be similar to those they currently use, so an equally plausible future scenario could include a slightly smaller volume of higher-concentration ingredients (or vice versa). Most company respondents reported purchasing less than 1 kg of omega-3 ingredients currently, but in contrast, the median respondent expected to purchase 100-1,000 kg five years from now.

Volume of omega-3 ingredients (kg) purchased in the past year versus anticipated annual purchase volume in five years



Figure 32. Current and anticipated volumes of omega-3 ingredients. Q13. How many kilograms of omega-3 ingredients has your company or lab purchased in the past year? Q44. How many kilograms of omega-3 ingredients would you anticipate needing to purchase per year in 5 years' time? (Please assume that the omega-3 concentration of these ingredients is similar to what you purchase today.) Note: The options provided on a survey were on a log scale, so the magnitude of the shift is larger than it appears visually.

Anticipated challenge in sourcing ingredients

Company respondents were asked whether omega-3 ingredient sourcing was expected to represent a minor, medium, or major challenge in five years. We provided the same definitions as in the earlier question about current challenges:

Minor challenge

Sourcing these ingredients introduces occasional challenges or headaches (e.g., prices are higher than we would like, or we sometimes run into delays with orders).

Medium-size challenge

Sourcing these ingredients introduces substantial issues (e.g., prices are high enough to substantially affect the cost of production, or we sometimes have to delay production runs or experiments due to issues with ingredient orders, but these issues are not prohibitive).

Major challenge

Issues with sourcing these ingredients seriously impacts our ability to conduct our experiments or to produce products at the scale, price, and quality that we otherwise could.

Most companies expected at least a minor challenge, with the "Medium-size" option receiving the most responses, but also a somewhat even split between "Minor," "Medium-size," and "Major" (n=20, Figure 33). This represents a substantial shift compared to the responses to the earlier question about the magnitude of the current challenge (n=21, counting only responses from company representatives), where most respondents selected either "Not a challenge" or "Medium-size challenge."

Current versus anticipated challenge in sourcing omega-3s



Figure 33. Current and anticipated magnitude of the challenge associated with sourcing omega-3 ingredients. Q16. How big of a challenge would you say sourcing of omega-3s, including EPA and DHA, is for your lab or company currently? Q45. Assuming the same rate of growth as in the last question, how big of a challenge would you anticipate that sourcing of omega-3s, including EPA and DHA, might be within the next 5 years? Assume that the omega-3 ingredient industry is similar to that of today.

Considering the higher targets for future omega-3 and EPA/DHA content, along with potential future growth in product volumes, the expectation of increased ingredient volumes and sourcing challenges is not surprising. However, this question assumed that the omega-3 ingredient supply would be comparable to today's market. This question was intended to measure companies' predictions of where they themselves would be in five years, not their level of optimism about the trajectory of the omega-3 industry. Thus, the correct interpretation of this result is not that alternative protein companies predict that they will face greater challenges in the future in sourcing omega-3 ingredients. Rather, these companies predict that they will face greater challenges in sourcing these ingredients unless there are substantial advances in upscaling and lowering costs in the omega-3 ingredient industry. If one is optimistic about the future growth of omega-3 ingredients derived from algae, plant molecular farming, precision fermentation, and other alternative sources, it is also reasonable to be optimistic that omega-3 ingredient supply will not represent an insurmountable barrier to the goal of nutritionally equivalent alternative meat and seafood at scale.

Open-ended responses (Q46) underscored the fact that many companies anticipate substantial challenges, such as price and volume availability, in sourcing omega-3 ingredients:

Q46. Please elaborate on the extent and nature of your anticipated challenges when sourcing omega-3-containing ingredients.

"Market demand could outstrip supply for solely algae sourced omega without major regulatory approval and successful commercialization from other potential sources (recombinant, cell-free)."

"Our current sense is that omega-3 price will be a larger issue than available volumes. This remains one of the most expensive inputs for us, without a clear path to cost reduction currently."

The scale of the challenge

To estimate the potential demand for omega-3 ingredients by the future alternative seafood industry, we started by looking at the ALA, EPA, and DHA content of conventional seafood. Because omega-3s are not produced by animal cells, and long-chain omega-3s are not produced by terrestrial plants, we generally expect alternative protein products to rely on external sources of these ingredients. For this calculation (see Appendix for details), we focused on U.S. imports, simply because reliable numbers were available (NOAA 2020). We also used published estimates of the fatty acid content (FAO 2016; USDA ARS 2019) of various seafood archetypes (where an "archetype" is a food-relevant seafood category such as salmon or trout). Based on these two inputs, we calculated a volume-weighted average ALA, EPA, and DHA content for U.S. seafood imports. Essentially, this second set of numbers represents how much of each fatty acid would be needed as an ingredient to produce a given volume of "average" seafood.

Notably, the EPA and DHA contained within U.S. seafood imports mostly represent a few specific archetypes, salmon and shrimp/prawns being chief

among them (Figure 34A). The prominence of salmon is driven by both its high EPA and DHA content (the highest of all archetypes analyzed here, with 1.8 g EPA+DHA per 100 g, though mackerel (1.28 g/100 g) and trout (1.49 g/100 g) had comparable amounts), and its high import volume (second after shrimp/ prawns). The high overall score of shrimp/prawns was driven mainly by their status as the most-imported archetype, even with its somewhat low EPA and DHA content (0.23 g/100 g). Nearly all ALA in U.S. seafood imports is from salmon.

Though the omega-3 content of terrestrial meat is substantially lower, it may still be significant when thinking about production at larger scales. Therefore, we performed a similar calculation starting from estimates of worldwide poultry, pork, and beef/ buffalo production and corresponding nutrition profiles (FAO–with major processing by Our World in Data 2023; USDA ARS 2019). According to this estimate, the majority of EPA and DHA contained in meat is from poultry (Figure 34A), due to a combination of higher EPA and DHA content (0.007 g/100 g) and higher production volumes compared to beef/buffalo (0.003 g/100 g) or pork (0.002 g/100 g). In contrast, the majority of ALA in meat is in pork.



Estimated total ALA, EPA, and DHA content of conventional seafood (U.S. imports) and meat (global production)

Figure 34. Estimated total ALA, EPA, and DHA content in 2020 U.S. seafood imports (A) and in 2021 total world meat production (B), colored by seafood or meat archetype. Note: Values for ALA concentration were unavailable for some seafood archetypes, meaning that this number may be a slight underestimate. As the top three most-imported archetypes (salmon, shrimp/prawns, and tuna [not specified], which account for 65% of the imports) all had ALA values available, we assume that this difference is fairly minor.



We extrapolated from these estimates to chart the relationship between the size of the global alternative meat and seafood market and the industry's future demand for EPA and DHA as ingredients. It is important to note that the mix of seafood archetypes consumed globally is certainly different from that represented by U.S. imports. Our estimate relies on the assumption that the average EPA and DHA content of seafood globally is roughly similar to that of U.S. imports, even if the archetypes consumed are quite different. We also assumed that alternative meat and seafood products will target products with similar EPA and DHA content to their conventional counterparts. To provide context for these estimates, we converted third-party estimates of the future growth of the alternative protein industry from dollars to tonnes (see Appendix for details). To our knowledge, only one published report (Adam et al. 2023) includes separate estimates for alternative seafood, and this report provides long-term estimates only in the case of cultivated products. Therefore, we used their estimates for cultivated seafood as a proxy for alternative seafood as a whole, with the understanding that plant-based and fermentationderived products may contribute to additional omega-3 ingredient demand. For alternative meat, we used a separate industry report (Klerk et al. 2021) that provides both short-term (2030) and longterm (2050) projections, which are inclusive of plantbased, fermentation-derived, and cultivated meat.



Forecasts for global alternative protein industry market size

Figure 35. Third-party projections of future market sizes for cultivated seafood (Adam et al. 2023), cultivated meat (Adam et al. 2023), and alternative meat (Klerk et al. 2021) in 2030 and 2050. Dollar amounts are reproduced directly from the original sources, and have been converted to tonnes for display purposes (see Appendix for details).

How much EPA and DHA will the alternative seafood industry need?

Focusing first on short-term scenarios, we estimate that if global cultivated seafood production were to reach the published projection (Adam et al. 2023), 267–1016 tonnes of EPA and 489–1857 tonnes of DHA would be required by 2030 (Figure 36). For context, this corresponds to approximately 0.075– 0.29 percent of 2018 global seafood production (FAO 2020). The total volume of algal oil produced for the food ingredients market in 2022 was estimated at 5,327 tonnes, not including algal oil used for aquafeed or other animal feed (GOED 2023). Assuming a combined EPA/DHA concentration of 41 percent (Kleiner, Cladis, and Santerre 2015), this would correspond to 2,184 tonnes of food-grade algal EPA/DHA (Figure 36-Figure 38). Around 50 percent of food-grade algal oil ingredients are currently used in infant formula, with most of the remainder going to either food and beverages or dietary supplements (GOED 2023).

For cultivated seafood to reach the 2030 projection with algal oil as its main omega-3 source (assuming no changes in algal oil use by other sectors), food-grade algal EPA and DHA production would need to increase by approximately 35–132% relative to 2022.



Projected ALA, EPA, and DHA demand as a function of alternative seafood production (short-term scenario)

Figure 36. Estimated demand for ALA, EPA, and DHA as ingredients for alternative seafood products as a function of global alternative seafood production. This estimate assumes that the average ALA, EPA, and DHA content of alternative seafood products will be similar to that of 2020 U.S. seafood imports. Shaded regions indicate third-party projections (Adam et al. 2023) of the cultivated seafood market size (converted from dollars to tonnes) and the dashed line indicates an estimate (GOED 2023) of the current size of the algal ingredient market (representing the EPA and DHA content of these ingredients only). The x-axis represents the range of scenarios in which alternative seafood is equal to up to ~0.56% of 2018 seafood production (FAO 2020).

Disclaimer: Market size projections are provided for context only, and do not necessarily indicate GFI's endorsement. Such projections can be useful tools, but we recommend interpreting them with caution.

Approaching the problem from the perspective of dietary recommendations for EPA and DHA gives an approximately similar result. We calculated that the EPA+DHA content in 0.56 percent of 2018 world seafood (the right-most extent of the x-axis in Figure 36) was approximately 5,608 tonnes. To provide 500 mg EPA+DHA per person per day (Givens and Gibbs 2008) to 0.56 percent of the 2018 world population of 7.66 billion people (Data Commons, n.d.) would require 3,924 tonnes of EPA+DHA. It is important to note that recommendations for EPA and DHA vary considerably, ranging from 250–500 mg for healthy adults but up to several times that for individuals in certain groups (Hjalmarsdottir 2019). Longer-term scenarios would require even more EPA and DHA ingredients. The amount of EPA and DHA ingredients required to reach the 2050 projections (Adam et al. 2023) (whether lower- or higher-end) greatly exceeds the size of the current algal EPA/ DHA ingredient market (GOED 2023) (Figure 37). This means that for cultivated seafood to reach the 2050 projection (assuming the same caveats as above), food-grade algal EPA and DHA production would need to increase by approximately 429–1717 percent relative to 2022.

Projected ALA, EPA, and DHA demand as a function of alternative seafood production (medium-term scenario)



Figure 37. Estimated demand for ALA, EPA, and DHA as ingredients for alternative seafood products as a function of global alternative seafood production. This estimate assumes that the average ALA, EPA, and DHA content of alternative seafood products will be similar to that of 2020 U.S. seafood imports. Shaded regions indicate third-party projections (Adam et al. 2023) of the cultivated seafood market size (converted from dollars to tonnes) and the dashed line indicates an estimate (GOED 2023) of the current size of the algal ingredient market (representing the EPA and DHA content of these ingredients only). The x-axis represents the range of scenarios in which alternative seafood is equal to up to ~4.5% of 2018 seafood production (FAO 2020).

Disclaimer: Market size projections are provided for context only, and do not necessarily indicate GFI's endorsement. Such projections can be useful tools, but we recommend interpreting them with caution.

How much EPA and DHA will the alternative meat industry need?

In contrast to seafood, the EPA and DHA volumes needed to match both the low-end and the highend 2030 projections by Klerk et al. (2021) with nutritionally equivalent alternative meat products are within current production levels of food-grade algal sources (Figure 38). This is true even with the much greater projected volumes of alternative meat than cultivated seafood (please note that Figure 37 and Figure 38 have different x-axes). For alternative meat to reach the 2050 projection (assuming the same caveats as above and ignoring the needs of alternative seafood), food-grade algal EPA and DHA production would need to increase by approximately 111–222 percent relative to 2022.

Projected EPA and DHA demand as a function of alternative meat production



Figure 38. Estimated demand for EPA and DHA as ingredients for alternative meat products as a function of global alternative meat producton. This estimate assumes that the average EPA and DHA content of alternative meat products will be similar to that of conventional meat products produced in 2021. Shaded regions indicate third-party projections (Klerk et al. 2021) of the alternative meat market size (converted from dollars to tonnes), and the dashed line indicates an estimate (GOED 2023) of the current size of the algal ingredient market (representing the EPA and DHA content of these ingredients only). The x-axis represents the range of scenarios in which alternative meat is equal to up to ~43% of 2021 meat production (FAO–with major processing by Our World in Data 2023).

Disclaimer: Market size projections are provided for context only, and do not necessarily indicate GFI's endorsement. Such projections can be useful tools, but we recommend interpreting them with caution.

Could ALA be a future bottleneck?

Nutritionally equivalent alternative terrestrial meat products would require much more ALA than EPA or DHA (Figure 39). It is therefore worth asking whether ALA represents a potential future bottleneck. Worldwide flax (linseed) production was approximately 3.4 million tonnes in 2020 (FAO 2023). With an ALA (PUFA 18:3) content of 22.8 percent, this would translate to 775,200 tonnes of ALA from flax alone. Thus, for alternative terrestrial meat to reach the third-party projections (Klerk et al. 2021) for 2050 with nutritionally equivalent products, less than 10 percent of the world's flax crop would be required. In reality, ALA is available from a variety of plant sources (e.g., <u>7.45% in canola oil</u>), so even less pressure on a single crop as a source would be expected.



Projected ALA, EPA, and DHA demand as a function of alternative meat production

Figure 39. Estimated demand for ALA, EPA, and DHA as ingredients for alternative meat products as a function of global alternative meat production. This estimate assumes that the average ALA, EPA, and DHA content of alternative meat products will be similar to that of conventional meat products produced in 2021. Shaded regions indicate third-party projections (Klerk et al. 2021) of the alternative meat market size (converted from dollars to tonnes) and the dashed line indicates an estimate (FAO 2023) of the current-day size of the flax ingredient market (representing 10% of the ALA content of these ingredients). The x-axis represents the range of scenarios in which alternative meat is equal to up to ~43% of 2021 meat production (FAO-with major processing by Our World in Data 2023).

Disclaimer: Market size projections are provided for context only, and do not necessarily indicate GFI's endorsement. Such projections can be useful tools, but we recommend interpreting them with caution.

In summary, the supply of algal-derived EPA and DHA has the potential to become a bottleneck for alternative seafood in the next few years, depending on the relative rates of growth of both of these nascent industries. For longer-term scale-up of alternative seafood products, substantial growth in alternative EPA and DHA sources—whether from algae or elsewhere—will be necessary. Assuming that alternative terrestrial meat producers aim to meet or exceed the EPA and DHA content of conventional products in the long term, these ingredients could become a bottleneck for alternative meat as well, though the scale and urgency of the challenge are lower. Even at longer time scales, we do not expect ALA production volumes to represent a major bottleneck for either product type.

Knowledge gaps, obstacles, market drivers, and motivations

The final survey section asked a series of open-ended questions about knowledge gaps related to omega-3s in alternative meat and seafood, obstacles preventing omega-3 fortification in alternative meat and seafood products, key market drivers, and companies' motivations for adding omega-3s to their products. This section summarizes the key knowledge from those responses.

What don't we know?

Participants were asked for their perspectives on key knowledge gaps related to omega-3s in alternative meat and seafood (n=16).

Q50. What do you perceive as the key knowledge gaps related to omega-3s for alternative meat and seafood? What research or other efforts would you like to see within the next 5 years to address these gaps? (Examples of knowledge gaps could include the environmental or economic feasibility of particular omega-3 production methods, the propensity for particular cell types to take up omega-3s in culture, or consumer perceptions of omega-3-containing foods.)

Oxidation and stability: One frequently mentioned theme among the listed knowledge gaps was the stability or shelf life of ingredients or products (four mentions). One respondent specifically mentioned stability at room temperature. The related theme of oxidation or antioxidants was mentioned twice, with a total of five responses that mentioned at least one of these themes. This is consistent with the earlier finding (see page 16) that shelf life and off-flavors were among the top challenges.

Human health and bioavailability: Human health was mentioned three times, specifically diseases caused by omega-3 deficiency, verifiable claims related to omega-3 health effects, and distinguishing between good and bad fatty acids from a health perspective. Two responses mentioned bioavailability, one of which mentioned the need to better understand the nature of any differences between animal-based and plant-based omega-3s.

Novel sources, cost, and sustainability: A number of responses focused on omega-3 sources, including two that specified natural sources. One of these suggested looking for sources available in active germplasm banks, and the other mentioned barriers to consumer acceptance of ingredients using genetic modification as a motivation for focusing on natural sources. One response listed recombinant sources of omega-3s as a knowledge gap. The sustainability (two mentions) and economics (four mentions) of omega-3 sources were also repeatedly mentioned.

Product formulation and omega-3 uptake and bioconversion: Responses also mentioned methods for introducing omega-3s into products (three mentions). Related to this, the uptake of omega-3s by cultivated cells from the culture media was also mentioned once. Two additional responses raised questions about the economic feasibility of relying on cultivated cells to produce their own omega-3s. Related to both the uptake and bioconversion themes, one response mentioned the need to better understand the relationship between the fatty acid inputs to cultivated cells and the fatty acid profile of the final product.

Finally, two responses mentioned that consumers themselves may have some knowledge gaps in their awareness of omega-3s.

What is holding us back?

Participants were also asked to describe the major obstacles to fortification with omega-3s. Sixteen participants responded.

Q51. What do you perceive as the major obstacles to wider omega-3 fortification of alternative meat or seafood products?

The cost of omega-3 ingredients was frequently mentioned as a major obstacle (seven mentions). Respondents were also concerned by the limited number of non-animal omega-3 ingredient sources, the limited supply of such ingredients, or the lack of suppliers (five mentions).

Off-flavors were mentioned four times, and oxidation or peroxidation were mentioned three times. Stability or shelf life was also mentioned three times. In total, eight responses mentioned some variation on the concepts of off-flavors, oxidation, stability, or shelf life.

Regulatory approval and formulation control were also mentioned in one response each. One respondent suggested that the ability of fish cells to convert plant-based omega-3s to EPA and DHA efficiently and economically might be an obstacle. Finally, another suggested that, at this early stage of the alternative protein industry, many companies are not thinking much about omega-3s and instead are focusing first on protein. This respondent stated that educating companies about omega-3s may be beneficial, and furthermore that cultivated fat companies can play a role.

What are the major market drivers?

Next, participants were asked for their perspectives on the major market drivers that could influence omega-3 ingredient use in alternative meat and seafood. Fourteen participants responded to this question.

Q52. What do you understand to be the major market drivers relevant to the use of omega-3 ingredients in alternative meat and seafood?

Overall, two clear themes stood out in the responses to this question. The first was health or nutrition (mentioned in eleven responses), and the second was consumer demand (seven responses). These two concepts were often mentioned together, for example, "Health conscious consumers," "Consumer demand for healthier food options," or "perceived health benefits, uniqueness of seafood/fish as a main protein with omega-3 and consumer desire for omega-3 when purchasing fish." Two responses additionally mentioned the positive impacts of omega-3s-or of fatty acid profiles more broadlyin determining product flavor. Availability and/or costs were mentioned twice, while sustainability, "processed foods," and "clean labels" were each mentioned once.

While respondents were not explicitly asked whether they expected their identified market drivers to push toward or against the use of omega-3 ingredients, the health- and nutrition-focused answers were generally quite clear that respondents perceived this as a positive driver. This was true for consumer demand and flavor as well. The only clear negative drivers mentioned were cost and availability. The significance of sustainability and processed foods in this context was somewhat less clear, perhaps suggesting that respondents expect these to be important considerations for some consumers and for companies' success in marketing an omega-3containing alternative protein product to depend in part on their success in these two areas.

Potential benefits of adding omega-3s to alternative meat and seafood products

Generally speaking, the benefits of adding omega-3s to alternative meat and seafood products can be classified into two major categories. First, to make a product that is just as good as its conventional equivalent, it will be necessary to replicate both the positive sensory qualities and the nutritional profile of conventional meat and seafood. Nutritional equivalence is likely to be especially important in the case of alternative seafood and omega-3s—especially the long-chain varieties. Second, alternative protein products could be made even better than the original. This could provide additional motivation for consumers to choose them, above and beyond concerns about sustainability or other altruistic motivations. This could mean enhancing the omega-3 content of an already omega-3-rich product or introducing substantial amounts of omega-3s into a product that is normally low in these nutrients. Whether such <u>enhanced</u> <u>products</u> are advisable will depend on the technical feasibility of making these changes without negative sensory consequences, whether consumer demand exists for them, and regulatory agencies' approach to fortified products.

Why are respondents' companies using omega-3s?

Finally, participants representing companies were asked to describe their own company's motivations for fortifying their products with omega-3 ingredients, either now or in the future. This question received eleven responses, and the overall themes discussed broadly mirrored those of the previous question about market drivers.

Q53. If you are fortifying your products with omega-3s or planning to in the future, why?

A clear theme was that companies want the nutrition profiles of their products to be equivalent to those of conventional meat or seafood products. For example, one respondent wrote, "We believe there is a consumer expectation around nutritional equivalence in cultivated seafood products, and that this extends to omega-3 content." Six responses mentioned equivalence to conventional products, with five of these mentioning nutritional equivalence. The broad concepts of nutrition or health were mentioned in nine responses, including one respondent who mentioned the importance of omega-3s for pet health.

The desire to appeal to consumers or to produce a premium product was also mentioned in four responses. Only two companies who answered this question were exclusively focused on terrestrial alternative meat products. Interestingly, both of these responses mentioned premium products or consumer adoption as well as nutrition or health, and neither mentioned equivalence to conventional products. One respondent wrote: "Omega-3s aren't a short-term target for us due to the technical and regulatory challenges involved. In the medium term I think nutrition will be key for driving consumer adoption, although omega-3s are not typically associated with [terrestrial meat] products." This is consistent with the earlier finding that companies focused on terrestrial alternative meat do not appear to be focusing on omega-3s in their current products, but show some interest in pursuing equivalent or higher omega-3 content in their future products (see page 40).





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Appendix

Survey methodology

Survey 1

The survey was constructed using an Airtable form. The list of questions is reproduced below. The survey was distributed to alternative meat and seafood companies and researchers through both direct outreach to individuals in GFI's network as well as through social media posts and newsletters. Potential respondents were also offered the option of a structured interview over video call, with a GFI representative filling out the survey on their behalf. The survey was originally scheduled to run from September through November of 2023, and was extended through December of 2023. Outreach included both alternative meat and alternative seafood companies, with a priority placed on the latter.

After noticing that there were fewer than expected responses from alternative meat companies, we constructed a shorter version of the survey that consisted of only Section 1 (Q1-12), Q33, Q35-36, Q43, Q47-49, and Q53-54. As this version of the survey was targeted at companies only, we restricted answers to Q7 to allow only that option to be selected, and added a note instructing researchers to use the full survey. Answers to the short survey fed into the same dataset as the longer version.

Survey 2

We also added several questions (reproduced below) to GFI's annual company survey in an effort to get a more representative picture of omega-3 use in the alternative meat and seafood industry. The omega-3-specific questions (Q2-215 through 02-221) were included only for respondents who identified themselves as either alternative protein manufacturers or suppliers primarily involved in alternative proteins in an earlier question. Results of several demographic questions presented earlier in the survey are also presented in this report. The full survey dataset included 109 alternative protein companies and 62 suppliers primarily involved in alternative proteins. Out of these, twenty answered at least one of the questions about omega-3s and are included in the data presented here.

Data cleaning and visualization

In a small number of cases, manual adjustments were made to survey responses, for example by removing "N/A" or irrelevant free-text responses to questions asking for clarification following an "Other" response, by merging duplicate responses from multiple representatives of the same company, or by adding responses to multiple choice questions that were not initially filled in but that seemed to be addressed by a respondent's free-text answers. Where necessary, these were clarified with the survey respondent before making any changes.

The datasets were exported as CSV files, and analysis and visualization were performed using Python and Matplotlib in a Jupyter notebook. In some cases, labels shown on graphs were abbreviated relative to the options provided in the survey. The exact wording included in the survey can be found in the question list.

Question list (survey 1)

*indicates required questions

Section 1 — demographics

- *By completing this form, you confirm that you agree to the processing of your personal data by GFI as described in the Privacy Notice (https:// gfi.org/privacy-policy/)
- 2. *Full name
- 3. *Company, University, or other Affiliation
- 4. *Position (E.g., CTO, Director of Product Development, Professor)
- 5. *Email
- 6. Survey results will be available to participants at no cost. Would you like to receive the aggregated results when they are available? (Yes | No)
- *Which of these best describes you/your company? (Alternative protein company | Academic researcher)
- 8. *Type of product your company or lab focuses on (Plant-based | Fermentation (Biomass) | Fermentation (Precision) | Cultivated | Hybrid) Help text: Please select multiple options only if working on hybrid products (e.g., if your products contain both cultivated cells and plant protein). If you are working on multiple types of products separately (e.g., if you produce separate cultivated and plant-based products), please fill out the rest of the survey in relation to just one. You may submit a separate response for the other product type if you like.
- *Species/product focus (Salmon | Tuna | Other finfish (please specify) | Shrimp | Lobster | Crab | Other crustacean (please specify) | Other seafood (please specify) | Chicken | Beef | Pork | Lamb/mutton | Turkey | Other terrestrial animal (please specify))

Help text: If you work on multiple species, please fill out the rest of the survey in relation to just one (unless the omega-3 content of all products is fairly similar). You may submit a separate response for your other products if you like.

- Please specify the species you're focusing on Help text: If the exact species is confidential, please share as specifically as you can.
 Conditional field: Shown if any of the "Other" options were checked in response to the previous question.
- 11. How would you describe the stage at which your company is currently operating?
 (R&D | Prototype | Pilot | Demonstration scale | Industrial scale)
 Conditional field: Shown if the respondent indicated in response to Q7 that they are from an alternative protein company.
- 12. Are there any further details or clarifications you'd like to share in relation to your answers in this section?

Section 2 — Volumes & challenges

Help text: This section will ask for details about your current use of omega-3-containing ingredients and about the extent and nature of the challenges you face when it comes to sourcing and using these ingredients.

- 13. How many kilograms of omega-3 ingredients has your company or lab purchased in the past year? (<1 | 1-10 | 10-100 | 100-1,000 | 1,000-10,000 | 10,000-100,000 | >100,000)
- 14. For the ingredients mentioned in the question above, what is the total omega-3 content? (<10% | 10-20% | 20-30% | 30-40% | 40-50% | 50-60% | 60-70% | 70-80% | 80-90% | >90% | Unknown)
- 15. For the ingredients mentioned in the question above, what is the total content of EPA and DHA combined? (<10% | 10-20% | 20-30% | 30-40% | 40-50% | 50-60% | 60-70% | 70-80% | 80-90% | >90% | Unknown)
- 16. How big of a challenge would you say sourcing of omega-3s, including EPA and DHA, is for your lab or company currently? (Not a challenge | Minor challenge | Medium-size challenge | Major challenge)

Help text: Minor challenge: Sourcing these ingredients introduces occasional challenges or headaches (e.g., prices are higher than we would like, or we sometimes run into delays with orders). Medium-size challenge: Sourcing these ingredients introduces substantial issues (e.g., prices are high enough to substantially affect the cost of production, or we sometimes have to delay production runs or experiments due to issues with ingredient orders, but these issues are not prohibitive). Major challenge: Issues with sourcing these ingredients seriously impacts our ability to conduct our experiments or to produce products at the scale, price, and quality that we otherwise could.

- 17. What specific challenges are you facing when it comes to sourcing omega-3s? (Price | Long lead times | Regulatory barriers | Trouble finding suppliers | Inconsistent supply | Inconsistent quality | Off-flavors | Shelf life | EPA/DHA ratio | Insufficient omega-3 content | Insufficient EPA/ DHA content | Purity | Other (please specify)) Help text: Please select all that apply.
- Please specify what other challenges you are facing:
 Conditional field: Shown if "Other" selected in response to the previous question.
- 19. Please elaborate on the extent and nature of your challenges when sourcing omega-3-containing ingredients.
- 20. Do you purchase EPA and DHA or produce them in-house? (Purchase | In-house | Some of each)
- 21. Approximately what percentage of the EPA and DHA your lab or company uses do you produce in-house? *Conditional field:* Shown if "Some of each"

selected in response to the previous question.

22. Where are your EPA and DHA sources coming from? (Algae (native expression) | Other marine microorganism (native expression) | Recombinant expression in microorganisms | Recombinant expression in plants | Cell-free systems | Fish oil or other animal source | Other (please specify))

Help text: Please select all that apply.

- 23. Please specify what other sources you are using: **Conditional field:** Shown only if "Other" selected in response to the previous question.
- 24. What additional EPA and DHA sources or production mechanisms are of most interest to your company or lab? (Algae (native expression) | Other marine microorganism (native expression) | Recombinant expression in microorganisms | Recombinant expression in plants | Cell-free systems | Fish oil or other animal source | Other (please specify))
- 25. Please specify what other sources you are most interested in:
 Conditional field: Shown only if "Other" selected in response to the previous question.
- 26. In what form(s) are you currently sourcing EPA and DHA? (Free fatty acid | Triglyceride | Phospholipid | Ethyl ester | Glycolipid | Wax ester | Other (please specify))
 Help text: Please select all that apply.
- 27. Please specify what other forms of EPA and DHA you are using:
 Conditional field: Shown only if "Other" selected in response to the previous question.
- In what form(s) are you most interested in sourcing EPA and DHA? (Free fatty acid | Triglyceride | Phospholipid | Ethyl ester | Glycolipid | Wax ester | Other (please specify)) Help text: Please select all that apply.
- 29. Please specify what other forms of EPA and DHA you are interested in: **Conditional field:** Shown only if "Other" selected in response to the previous question.
- 30. Do you use encapsulated omega-3 ingredients? (Yes | No | No, but I would be interested in trying such ingredients)
 Help text: This question refers to ingredients that are encapsulated within a protective material to protect the fatty acids from oxidation and rancidity.
- 31. What type(s) of omega-3 ingredients are compatible with your needs? (Concentrate | Refined oil | Minimally processed oil)
 Help text: Please select all that apply.

32. Please share any further details related to omega-3 (including EPA and DHA) ingredient volumes and current challenges that aren't captured by your answers above. If you'd like to provide any clarifications related to your answers above, please do so here.

Section 3 — Current products

Help text: This section will ask about your current products. If you already have an alternative meat or seafood product on the market, please answer with respect to that product. If your products are still under development or awaiting regulatory clearance, please answer with respect to your current product prototypes.

Conditional section: All fields in this section were shown only if the respondent indicated in response to Q7 that they are from an alternative protein company.

- 33. My answers in this section reflect: (Current products on the market | Current prototypes (with characterization | Current prototypes (target)) *Help text: "With characterization" means that you have produced prototypes and have characterized their fatty acid profile. "Target" means that you are at an earlier stage in the product development process and are answering with respect to the fatty acid profile you're aiming for in your current prototypes, but haven't yet confirmed that this has been achieved.*
- 34. How many kilograms of your product or prototype have you produced in the past year? (<1 | 1-10 | 10-100 | 100-1,000 | 1,000-10,000 | 10,000-100,000 | 100,000-1 MM | 1 MM - 10 MM | >10 MM)
- 35. Compared to conventional equivalents (e.g., conventional salmon if your company makes plant-based salmon), what is the total omega-3 content of your company's current products or prototypes? (Omega-3 content is not an explicit consideration | Lower omega-3 content (by more than 10% | Equivalent omega-3 content (±10%) | Higher omega-3 content (by more than 10%))

- 36. Compared to conventional equivalents (e.g., conventional salmon if your company makes plant-based salmon), what is the EPA/DHA omega-3 content of your company's current products or prototypes? (EPA/DHA content is not an explicit consideration | Lower EPA/DHA content (by more than 10% | Equivalent EPA/DHA content (±10%) | Higher EPA/DHA content (by more than 10%))
- 37. How does your company currently introduce EPA and DHA into your cultivated products or prototypes? (EPA and DHA are added to the culture media | EPA and DHA are incorporated into the scaffold material | EPA and DHA are added in a processing step after cell harvest | EPA and DHA are produced by co-cultured animal cells | EPA and DHA are produced by co-cultured non-animal cells | The cells we use to cultivate our product produce their own EPA and DHA | We don't do anything to introduce EPA or DHA | Other (please specify)) Help text: Please select all that apply. Conditional field: Shown only if "Cultivated" was selected in response to Q8.
- 38. Do you add shorter-chain omega-3s intended to serve as a substrate for conversion to EPA and DHA? (No | No, our culture system can produce EPA and DHA from non-omega-3 building blocks) Conditional field: Shown only if any of "EPA and DHA are produced by co-cultured animal cells," "EPA and DHA are produced by co-cultured nonanimal cells," or "The cells we use to cultivate our product produce their own EPA and DHA" were selected in response to the previous question.
- 39. Please specify how you introduce EPA and DHA: *Conditional field:* Shown only if "Other" selected in response to Q37.
- 40. How does your company currently introduce omega-3s (besides EPA and DHA) into your cultivated products or prototypes? (Omega-3s are added to the culture media | Omega-3s are incorporated into the scaffold material | Omega-3s are added in a processing step after

cell harvest | Omega-3s are produced by co-cultured animal cells | Omega-3s are produced by co-cultured non-animal cells | The cells we use to cultivate our product produce their own omega-3s | We don't do anything to introduce omega-3s besides EPA and DHA | Other (please specify))

Conditional field: Shown only if "Cultivated" was selected in response to Q8.

- 41. Please specify how you introduce omega-3s besides EPA and DHA:
 Conditional field: Shown only if "Other" selected in response to the previous question.
- 42. Have the cell type(s) used to produce omega-3s (including EPA and DHA) within your culture system been modified to have this ability? (Yes, the cultivated cells are modified to produce omega-3s | Yes, the co-cultured cells are modified to produce omega-3s | Yes, the cocultured cells are modified to produce omega-3s) Help text: This question refers to approaches in which either the cultivated cells (e.g., the animal muscle or fat cells) or an additional co-cultured cell type (either animal or non-animal) serves as a source of omega-3s within the culture system. This may include both 1) approaches where such cells are used to produce omega-3s from nonomega-3 precursors as well as 2) those where cells are used to produce EPA and DHA from shorter-chain omega-3s.

Conditional field: Shown only if any of "EPA and DHA are produced by co-cultured animal cells," "EPA and DHA are produced by co-cultured nonanimal cells," or "The cells we use to cultivate our product produce their own EPA and DHA" were selected in response to Q37, and/or if any of "Omega-3s are produced by co-cultured animal cells," "Omega-3s are produced by co-cultured non-animal cells," or "The cells we use to cultivate our product produce their own omega-3s" were selected in response to Q40.

43. Please share any further details related to the omega-3 content of your current products or prototypes that aren't captured by your answers above. If you'd like to provide any clarifications related to your answers above, please do so here.

Section 4 — Future projections

Help text: This section will ask about where you anticipate your company's products will be in 5 years. For all questions in this section, please assume an optimistic but realistic rate of growth for your company. We recognize that future projections come with a great deal of inherent uncertainty and will present the results of this section's responses accordingly.

Conditional section: All fields in this section were shown only if the respondent indicated in response to Q7 that they are from an alternative protein company.

44. How many kilograms of omega-3 ingredients would you anticipate needing to purchase per year in 5 years' time? (<1 | 1-10 | 10-100 | 100-1,000 | 1,000-10,000 | 10,000-100,000 | >100,000)

Help text: Please assume that the omega-3 concentration of these ingredients is similar to what you purchase today.

45. Assuming the same rate of growth as in the last question, how big of a challenge would you anticipate that sourcing of omega3-s, including EPA and DHA, might be within the next 5 years? Assume that the omega-3 ingredient industry is similar to that of today. (Not a challenge | Minor challenge | Medium-size challenge | Major challenge)

Help text: Minor challenge: Sourcing these ingredients introduces occasional challenges or headaches (e.g., prices are higher than we would like, or we sometimes run into delays with orders). Medium-size challenge: Sourcing these ingredients introduces substantial issues (e.g., prices are high enough to substantially affect the cost of production, or we sometimes have to delay production runs or experiments due to issues with ingredient orders, but these issues are not prohibitive). Major challenge: Issues with sourcing these ingredients seriously impacts our ability to conduct our experiments or to produce products at the scale, price, and quality that we otherwise could.

46. Please elaborate on the extent and nature of your anticipated challenges when sourcing omega-3-containing ingredients.



- 47. Compared to conventional equivalents (e.g., conventional salmon if your company makes plant-based salmon), what is your target for the total omega-3 content of your company's future products within the next 5 years? (Omega-3 content is not an explicit consideration | Lower omega-3 content (by more than 10% | Equivalent omega-3 content (±10%) | Higher omega-3 content (by more than 10%))
- 48. Compared to conventional equivalents (e.g., conventional salmon if your company makes plant-based salmon), what is your target for the EPA/DHA omega-3 content of your company's future products within the next 5 years? (EPA/DHA content is not an explicit consideration | Lower EPA/DHA content (by more than 10% | Equivalent EPA/DHA content (±10%) | Higher EPA/DHA content (by more than 10%))
- 49. Please share any further details related to the omega-3 content of your planned future products or prototypes that aren't captured by your answers above. If you'd like to provide any clarifications related to your answers above, please do so here.

Section 5 — Obstacles & drivers

Help text: This section will ask about your perceptions of consumers' and companies' motivations related to omega-3s in alternative meat and seafood as well as challenges and knowledge gaps in this area. This will help us get a more comprehensive picture of how the industry is thinking about this challenge. Please feel free to provide as much or as little detail as you like. 50. What do you perceive as the key knowledge gaps related to omega-3s for alternative meat and seafood? What research or other efforts would you like to see within the next 5 years to address these gaps?

Help text: Examples of knowledge gaps could include the environmental or economic feasibility of particular omega-3 production methods, the propensity for particular cell types to take up omega-3s in culture, or consumer perceptions of omega-3-containing foods.

- 51. What do you perceive as the major obstacles to wider omega-3 fortification of alternative meat or seafood products?
- 52. What do you understand to be the major market drivers relevant to the use of omega-3 ingredients in alternative meat and seafood?
- 53. If you are fortifying your products with omega-3s or planning to in the future, why? **Conditional field:** Shown if the respondent indicated in response to Q7 that they are from an alternative protein company.
- 54. Anything else you wish we had asked about? *Help text:* If there's anything else you'd like to share that wasn't captured by the questions above, please do so here!

Question list (survey 2)

*indicates required questions

This list includes only those questions whose results are reported here or were used to determine whether a respondent would be presented with the omega-3related questions.

- 2-2. *Please select your organization type. If you fall into more than one category, please select the one that best describes your organization. (Food manufacturer | Investor | Restaurant/foodservice company | Retailer | Supplier (ingredient, equipment, etc.) | Service provider (CDMO, distributor, consultant, etc.))
- 2-3. Which of the following primarily describes your company? (If "Food manufacturer" selected in response to previous question: Alternative protein company (e.g., Impossible Foods, Beyond Meat, Meati, Upside Foods) | Large meat/protein company (e.g., Cargill, Tyson, Perdue Farms, Hormel Foods) | CPG company (e.g., Nestle, Kraft Heinz, Unilever, Conagra). If "Supplier" selected in response to previous question: Ingredient supplier | Equipment supplier | Other (please specify))
- 2-4. *Is your company involved in the alternative protein industry? (Yes, alternative proteins comprise a majority of my company's business | Yes, alternative proteins comprise less than half of my company's business | No) *Help text:* Alternative proteins are alternatives to meat, eggs, and dairy. Read more here: https://gfi.org/defining-alternative-protein/
- 2-5. In which alternative protein category(ies) is your business involved? (select all that apply) (Plant-based | Cultivated | Biomass fermentation | Precision fermentation | Plant molecular farming | Traditional fermentation | Other (please specify))
- 2-6. Company focus: (select all that apply) (Meat | Eggs | Dairy | Seafood | Oils and fats | Pet food | Infant nutrition | Ingredients and inputs | Equipment | Other (please specify))

- 2-25. In which production stage is your company currently operating? (Lab | Pilot (sampling and/or research batches) | Demo (process development for full-scale production) | Commercial (full-scale production))
- 2-215. Producing alternative meat, seafood, eggs, and dairy products with higher levels of omega-3 fatty acids (e.g., long-chain omega-3s like EPA and DHA) compared to their conventional counterparts is a possible benefit of alternative protein products. Which of the following most accurately describes your company's approach to such "omega-3-enhanced" products? (Part of our current product portfolio | Currently being developed | Under active consideration | Not a current priority, but a possibility depending on consumer demand | Not a current priority, and very unlikely to be a priority in the future)
- 2-216. Compared to conventional equivalents (e.g., conventional salmon if your company makes plant-based salmon), what is the total omega-3 content of your company's current products or prototypes? If your company produces multiple products and your answer differs depending on the product, please use the text box at the end of this section to provide further details. (Omega-3 content is not an explicit consideration | Lower omega-3 content (by more than 10%) | Equivalent omega-3 content (±10%) | Higher omega-3 content (by more than 10%))
- 2-217. Compared to conventional equivalents, what is the EPA/DHA omega-3 content of your company's current products or prototypes? (EPA/DHA content is not an explicit consideration | Lower EPA/DHA content (by more than 10%) | Equivalent EPA/DHA content (±10%) | Higher EPA/DHA content (by more than 10%))
- 2-218. My answers to the two questions above reflect: (Current products on the market | Current prototypes (with characterization: We have produced prototypes and have characterized their fatty acid profile) | Current

prototypes (target: We are at an earlier stage in the product development process and are answering with respect to the fatty acid profile we're aiming for in our current prototypes but haven't yet confirmed that this has been achieved))

- 2-219. Compared to conventional equivalents, what is your target for the total omega-3 content of your company's future products within the next 5 years? (Omega-3 content is not an explicit consideration | Lower omega-3 content (by more than 10%) | Equivalent omega-3 content (±10%) | Higher omega-3 content (by more than 10%))
- 2-220. Compared to conventional equivalents, what is your target for the EPA/DHA omega-3 content of your company's future products within the next 5 years? (EPA/DHA content is not an explicit consideration | Lower EPA/ DHA content (by more than 10%) | Equivalent EPA/DHA content (±10%) | Higher EPA/DHA content (by more than 10%))
- 2-221. Please share any further details related to the omega-3 content of your current or planned future products or prototypes that aren't captured by your answers above. If you'd like to provide any clarifications related to your answers above, please do so here.

Market sizing

To calculate the average ALA, EPA, and DHA content for seafood imported to the United States in 2020, we primarily used nutrition profiles from the uFiSh database (FAO 2016) and data on seafood imports from NOAA (NOAA 2020). The two datasets were aggregated according to archetype (e.g., salmon, trout) using GFI's ATLAS database. Because the uFiSh database lacked nutrition profiles for tuna, we also used FoodData Central (USDA ARS 2019) to find representative examples for the archetypes Tuna (Albacore/Skipjack), Tuna (Bluefin/Bigeye/Yellowfin), and Tuna (Not Specified). Production data from 2021 for chicken, pork, and beef/buffalo were accessed from Our World In Data, based on original data from the FAO (FAO-with major processing by Our World in Data 2023). Other archetypes were not included for the sake of simplicity, as these three made up the vast majority of global production. Nutritional data for chicken, pork, and beef were accessed from FoodData Central. Data were compiled in a Google Sheet and exported as a CSV file for analysis and visualization using Python/Matplotlib/Jupyter Notebook as described above.

For each archetype, the ALA, EPA, and DHA content were multiplied by the corresponding production or import value. The resulting values were summed over all archetypes, and this value was divided by the sum of the production or import values. The resulting ratio was taken to represent the "average" ALA/EPA/ DHA content of seafood or meat in that dataset, or in other words, the quantity (by mass) of ALA, EPA, or DHA present in a given quantity of "average" seafood or meat. This ratio was then multiplied by a given hypothetical future production volume of alternative meat or seafood to give an estimate of the corresponding volume of ALA/EPA/DHA ingredients needed.

Third-party projections of cultivated seafood and alternative meat market sizes (Adam et al. 2023; Klerk et al. 2021) were converted from dollars to tonnes by dividing by estimated retail prices for the relevant category. GFI tracks prices of alternative and conventional meat based on data from SPINS and Circana (Pierce et al. 2023). Conversion factors were chosen by selecting the most logical match within the available data. Specifically, 2030 terrestrial alternative meat, 2050 terrestrial alternative meat, and 2050 cultivated seafood prices were assumed to be similar to those of plant-based meat, conventional meat, and conventional seafood, respectively. Values for fresh and frozen seafood were averaged, given that sales of the two categories are approximately equal (FMI 2023). In lieu of reliable predictions of the price of cultivated meat and seafood in 2030, we assumed that prices would be double those of conventional meat and seafood.



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