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Review

New Sustainable Oil Seed Sources of Omega-3 Long-Chain Polyunsaturated Fatty Acids: A Journey from the Ocean to the Field

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Abstract: Omega-3 long-chain ($\geq C_{20}$) polyunsaturated fatty acids (ω3 LC-PUFA) play a critical physiological role in health and are nutritionally important for both humans and animals. The abundance of marine-derived resources of the health-benefitting ω3 LC-PUFA is either static or in some cases declining. This review focuses on the development and deregulation of novel oilseed crops producing ω3 LC-PUFA and their market applications. Genetic engineering of ω3 LC-PUFA into sustainable oilseed crops involving multiple-gene pathways to reach fish oil-like levels of these key nutrients has been extremely challenging. After two decades of collaborative effort, oilseed crops containing fish oil-levels of ω3 LC-PUFA and importantly also containing a high ω3/ω6 ratio have been developed. Deregulation of genetically engineered crops with such novel nutritional traits is also challenging and more trait-based regulations should be adopted. Some ω3 LC-PUFA-producing oilseed crops have been approved for large-scale cultivation, and for applications into feed and food. These genetically engineered oilseed crops can and will help meet the increasing market demand for aquaculture and human nutrition. These new oil seed sources of ω3 LC-PUFA offer a sustainable, safe, cost-effective, and scalable land-based solution, which can have critical and positive health, economic, and environmental impacts.

Keywords: aquafeed; DHA; DPA; EPA; ω3 LC-PUFA; oilseed crops; nutrition; regulatory; sustainability

Citation: Zhou, X.-R.; Yao, Z.J.; Benedicto, K.; Nichols, P.D.; Green, A.; Singh, S. New Sustainable Oil Seed Sources of Omega-3 Long-Chain Polyunsaturated Fatty Acids: A Journey from the Ocean to the Field. *Sustainability* **2023**, *15*, 11327. https://doi.org/10.3390/su151411327

Academic Editor: Vita Di Stefano

Received: 1 May 2023
Revised: 14 July 2023
Accepted: 17 July 2023
Published: 20 July 2023

1. Introduction

Omega-3 long-chain polyunsaturated fatty acids (ω3 LC-PUFA, defined as containing 20 or more carbon atoms), including eicosapentaenoic acid (EPA, 20:5ω3), docosapentaenoic acid (DPA, 22:5ω3), and docosahexaenoic acid (DHA, 22:6ω3), are beneficial to human health throughout the whole lifespan [1]. They are essential components of cell membranes important for cell function as well as precursors for biologically active signalling molecules in mammals.

DHA is one of the most important ω3 LC-PUFA. Sub-optimal levels of DHA in the human body are associated with an increased risk of several diseases [2]. Ghasemi Fard et al. [3] provided a comprehensive collection of evidence and a critical summary of the documented physiological effects of high DHA fish oils on human health. The positive effects of EPA and DHA have been reported across a range of degenerative and inflammatory disorders such as heart disease, stroke, rheumatoid arthritis, asthma and some cancers, diabetes mellitus, multiple sclerosis, dementia, and clinical depression [2,4,5]. EPA- and in particular DHA-rich oils are also important in infant nutrition, with DHA present in high concentrations in the brain and retina, and these two key LC-PUFA are important in the development, health, and enhanced functioning of these and other organs [6–8].

Similar to DHA and EPA, ω3 DPA is gaining increasing recognition and importance because of its unique properties. ω3 DPA is the precursor of many lipid mediators involved in the pro-resolution of inflammation with specific effects compared to other ω3 LC-PUFA [9]. The presence of ω3 LC-PUFA in human tissues and its relative abundance in human milk have long served as clues to its importance in human health. It is increasingly recognized as an important part of our diet. Numerous trials have demonstrated a clear link between ω3 DPA intake and better health, while multiple in vitro and in vivo studies have shown direct effects of ω3 DPA on inflammation, improved plasma lipid profile, and cognitive function [10,11]. Morin et al. [12] reported that ω3 LC-PUFA monoacylglycerides (MAG) were found to be better absorbed in cultured human colorectal cancer cells compared to the corresponding free fatty acids. Furthermore, that study demonstrated that ω3 DPA-MAG had increased anti-proliferative and pro-apoptotic effects, decreased cell proliferation and induced apoptosis, when compared to DHA-MAG and EPA-MAG. Recently, Ghasemi Fard et al. [13] summarised the physiological effect, delivery, fatty acid metabolism, and bioavailability of ω3 DPA.

ω3 LC-PUFA are also essential for fish development [14]. They are nutritionally important for the survival, growth, and general health of aquaculture species, particularly at the larval stage. Reduced accumulation of ω3 LC-PUFA in farmed fish also decreases the nutritional value of the final product [15,16].

The current principal sources of ω3 LC-PUFA for human consumption are wild-caught marine fish species, krill, and some algae. The increasing demand for these fatty acids has contributed in some regions to overfishing of many source species, generating a huge negative environmental impact [17]. In addition, global warming leading to an increase in water temperature, depending on the climate scenario and location, could result in a 10 to 58% loss of globally available DHA by 2100 [18]. The ω3 LC-PUFA in these fish species are accumulated up the food web, primarily originating from microalgae. While aquaculture is an alternative way to replace the wild fish stocks for human consumption of ω3 LC-PUFA, farmed fish need sustainable sources of ω3 LC-PUFA in their diet for their development and growth. This requirement constrains the impact that aquaculture per se can have on mitigating the decline in wild fish stocks, including in some cases due to unsustainable harvesting of wild fisheries.

Fermentation of microalgae containing ω3 LC-PUFA has also been seen as a potential solution in this area. However, growing microalgae heterotrophically has its own challenges, including energy consumption, the high capital investment required for large-scale fermentation facilities, reproducibility and consistency of production, efficiency of cell breaking, high production cost, and other factors. The development of algal-derived single cell oils will not be covered further in this review, and background on and an example of such research is covered in this Special Issue by Soudant et al. 2023 [19].

2. Development of Oilseed Crops with Fish Oil-like Levels of ω3 LC-PUFA

The above challenges have led to the exploration of alternative and sustainable approaches. Metabolic engineering of land-based oilseed crops to produce fish oil levels of ω3 LC-PUFA is one of the most striking and ambitious examples of such a strategy [20]. High oil yield and relatively low production costs of oilseed crops can provide an economic and sustainable production platform for oil containing ω3 LC-PUFA. For example, canola (*Brassica napus* L.) picks itself as a potential oil platform for EPA and DHA production. Canola seed yields have been reported up to 4 T/ha with 40–45% seed oil content. It has broad agronomic and geographic adaptation, considerable genetic resources, and substantially developed germplasms. These aspects make canola the second largest oilseed crop (behind soybean), producing 84.8 million metric tons (MMT) globally in 2022/23 [21] and representing an ideal vehicle for producing ω3 LC-PUFA.

LC-PUFA can be synthesised by two distinct pathways: the aerobic pathway utilizing fatty acid desaturases and elongases, and the anaerobic polyketide synthase (PKS) pathway [22]. The aerobic pathway uses sequential oxygen-dependent desaturation and elongation steps coupled with electron flow (Figure 1A). The same set of desaturases and elongases can synthesise ω6 DPA or ω3 DHA from the ω6 substrate linoleic acid (LA, 18:2ω6) or the ω3 substrate α-linolenic acid (ALA, 18:3ω3), respectively. The PKS pathway synthesises LC-PUFA directly from malonyl-CoA and acetyl-CoA without the need for oxygen for desaturation (Figure 1B) [23,24]. Such a complex pathway makes it challenging to engineer for achieving the desired high levels of specific end products. In the last two decades, these two distinct pathways have been introduced into oilseeds to produce ω3 LC-PUFA including EPA, ω3 DPA, and DHA [25–27]. The introduced aerobic pathway required genes for the five desaturation and/or elongation steps from ALA to DHA, while the introduced PKS pathway contained several genes for multiple domains (Figure 1). Most recently, production of ω3 docosatrienoic acid (DTA, 22:3ω3) in *B. carinata* was achieved by introducing a minimal single elongase from the plant *Eranthis hyemalis* that can elongate a wide range of PUFA, thus converting plant endogenous ALA to ω3 eicosatrienoic acid (ETA, 20:3ω3) then further converting ETA to DTA [28]. Generally, the production of ω3 LC-PUFA with the PKS pathway has resulted in only low levels of products [25]; however, efforts with the aerobic pathway have produced ω3 LC-PUFA at the same levels as found in wild fish oils [20,29].

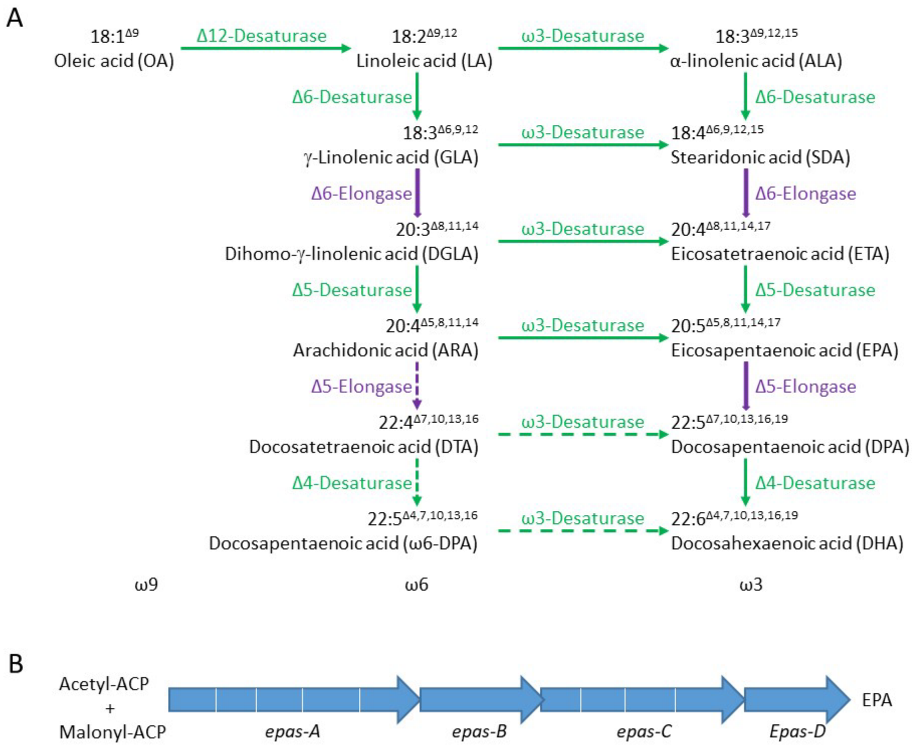

Figure 1. Biosynthetic pathways of LC-PUFA. (**A**) The aerobic pathway using specific desaturases (green) and elongases (purple) converts both ω6 LA or ω3 ALA to ω6 DPA or ω3 DHA, respectively. The dashed arrows are minor activity steps of the pathway in microalgae and engineered oil crops. (**B**) EPA polyketide synthase pathway from *Shewanellea oneidensis* [24] is composed of huge enzyme complexes with multiple domains as divided by bars.

Since the earlier demonstration of successful metabolic engineering of EPA or DHA production at low levels in yeast or seed oils [23,30–32], development of commercially sustainable oilseed crops with fish oil-like levels of ω3 LC-PUFA has been one of the main targets by a range of researchers and companies in the last two decades and longer in some cases. Previous reviews of the research and development on oilseed sources of ω3 LC-PUFA are available in [20,33–36].

Several of the research efforts have ceased, and/or not achieved what were seen as very difficult aims. The collaboration between CSIRO, the Grains Research and Development Corporation (GRDC), and Nuseed has continued and has successfully developed a genetically engineered canola, event NS-B5ØØ27-4, that produces ω3 LC-PUFA containing oil with levels of 9.7% DHA, 1% DPA, and 0.5% EPA [27]. An ongoing breeding program aims to further increase ω3 LC-PUFA levels in the oil. The project was initiated by CSIRO researchers in 1997, although it took several years to build momentum. The research team comprised: marine microalgae researchers, plant geneticists, plant breeders, marine oil chemists, food technologists, and other specialists. One key aspect was that the CSIRO research team accessed a unique selection of microalgae from the CSIRO-based Australian National Algae Culture Collection (http://www.csiro.au/ANACC (accessed on 1 June 2023)). The algae collection had been established at CSIRO for strategic research on algal chlorophyll and carotenoid pigments as applied in biological oceanographic research.

Another collaboration between BASF and Cargill has also generated a transgenic canola, event LBFLFK, that produces 0.3% DHA, 2% DPA, and 4% EPA in refined, bleached, and deodorized oil [37]. These two events (CSIRO-Nuseed and BASF-Cargill) will be covered in this paper, with emphasis on the former project. Key desaturase and elongase enzymes identified, validated (in yeast and plant models), and developed and used in the CSIRO-Nuseed project are listed in Table 1.

Table 1. DHA biosynthesis enzymes. In the isolation of an efficient synthesis pathway, key desaturase and elongase enzymes were isolated from strains held in the CSIRO-based Australian National Algae Culture Collection (http://www.csiro.au/ANACC (accessed on 1 June 2023)).

Enzyme	Conversion	Comment	References
Micromonas persilla Δ6-desaturase	18:3ω3 to 18:4ω3 (ALA to SDA)	Use of a marine microalgae Δ6-desaturase with a higher preference for ω3 substrate than ω6 substrate	[38]
Pyraminomas cordata Δ6-elongase	18:4ω3 to 20:4ω3 (SDA to ETA)	High conversion efficiency of SDA to ETA via Δ6-elongase	[39]
Pavlova salina Δ5-desaturase	20:4ω3 to 20:5ω3 (ETA to EPA)	Demonstrated the acyl-CoA desaturation ability	[39,40]
Pyraminomas cordata Δ5-elongase	20:5ω3 to 22:5ω3 (EPA to ω3 DPA)	Highly efficient Δ5-elongase targeted to maximise the elongation from EPA to ω3 DPA	[39]
Pavlova salina Δ4-desaturase	22:5ω3 to 22:6ω3 (ω3 DPA to DHA)	Demonstrated the acyl-CoA desaturation ability	[40]
ω3 desaturases from various sources	conversion of ω6 PUFA and ω6 LC-PUFA to ω3 PUFA and ω3 LC-PUFA	Results in very low amounts of ω6 fatty acids and contributed to the high ω3/ω6 ratio	[41]

Multiple attempts have been made to achieve the fish oil-like levels of ω3 LC-PUFA for commercialisation. The first consideration was to enhance the fatty acid flux from oleic acid (OA, 18:1ω9) to ω3 ALA by introducing a yeast Δ12-desaturase and ω3-desaturase, in addition to the endogenous Δ12-desaturase and Δ15-desaturase. ω3-Desaturases can convert a range of ω6 fatty acids including LA, γ-linolenic acid (GLA, 18:3ω6), dihomo-γ-linolenic acid (DGLA, 20:3ω6), arachidonic acid (ARA, 20:4ω6), docosatetraenoic acid (DTA, 22:4ω6), and docosapentaenoic acid (ω6 DPA, 22:5ω6) into the corresponding ω3 fatty acids with different substrate preferences [41]. The introduced ω3-desaturase maximally converts ω6 LA to ω3 ALA, thus making higher levels of ω3 substrate available for the biosynthesis pathway. The remaining low amount of LA is used for synthesis of the downstream ω6 LC-PUFA, which can also be converted to their ω3 counterparts by ω3-desaturase. This resulted in very low amounts of ω6 fatty acids and contributed to the high ω3/ω6 ratio [27] that is desired for both human and fish health.

The second consideration was to use a marine microalgae *Micromonas pusilla* Δ6-desaturase with a higher preference for the ω3 substrate than the ω6 substrate [38]. The combined effect of the enhanced fatty acid flux from OA to ALA, and the ω3 substrate preference of the Δ6-desaturase, led to the elevated production of ω3 fatty acids at the early steps of the biosynthetic pathway.

The third consideration was to use acyl CoA desaturases for subsequent steps in the pathway rather than phosphatidylcholine (PC) type desaturases to avoid excessive acyl shuffling between acyl-CoA and acyl-PC pools, as the fatty acid elongation occurs in acyl-CoA pools. Phylogenetic analysis of amino acid sequences showed that the *M. pusilla* Δ6-desaturase, *Pavlova salina* Δ5- and Δ4-desaturases used in the ω3 LC-PUFA containing canola event, NS-B5ØØ27-4, clustered with other demonstrated acyl-CoA desaturases. *M. pusilla* Δ6-desaturase has been demonstrated to have acyl-CoA desaturation ability [38]. *P. lutheri* Δ4-desaturase, from a very closed related species to *P. salina*, has also been shown to desaturate acyl-CoA substrates [42].

The fourth consideration was to use a highly efficient Δ5-elongase from the microalga *Pyramimonas cordata* to maximise the elongation from EPA to ω3 DPA. Earlier proof of concept work had expressed the DHA biosynthetic pathway containing *P. salina* Δ5-elongase in *Arabidopsis* and successfully produced DHA in seed oil, but only at low levels (<1%). The conversion rate of the Δ5-elongation step was the major bottleneck, with the efficiency lower than 20% [31]. The *P. cordata* Δ5-elongase showed much higher efficiency for elongating EPA to ω3 DPA in yeast cells [39] than the *P. salina* Δ5-elongase [43]. The superior conversion efficiency of *P. cordata* Δ5-elongase was confirmed to be as high as 90% in *Arabidopsis* seeds [44].

In addition, the large T-DNA vector consisting of seven genes in the DHA biosynthetic pathway plus a selection marker had been carefully designed with multiple seed-specific promoters. The promoter expression timing was an important factor to reduce accumulation of intermediate fatty acids. The direction of gene expression cassettes and the inclusion of non-coding spacers between cassettes was another consideration designed to maximise the gene expression levels. Finally, thousands of lines were created having stable inserts with relatively low copies of T-DNA. The selected elite canola event, NS-B5ØØ27-4, contains a multi-copy of the full transgene construct at one locus plus an extra partial T-DNA insertion at another locus effectively acting as a 'booster' for the full pathway with increased gene dosage.

In the case of canola event LBFLFK, gene dosage with multiple copies of alternate genes for the same enzymatic activity was a contributing factor [20]. The transgene cassette had a total of 12 genes for ω3 LC-PUFA biosynthesis, with two copies inserted in the canola chromosome. Increased gene dosage has also been applied in engineering EPA production in the yeast *Yarrowia lipolytica* by integrating five to seven copies of each desaturase or elongase gene, with a total number of 24 desaturase/elongase genes for maximised EPA accumulation [45]. These approaches collectively provide successful strategies for metabolic engineering that utilise complex multiple gene pathways.

In addition to canola, other oilseed crops such as *Camelina sativa* have been engineered for the production of ω3 LC-PUFA oil using a similar approach. A transgene cassette expressing five genes or seven genes for ω3 LC-PUFA biosynthesis from OA in *C. sativa* resulted in 24% EPA or 11% EPA and 8% DHA in seed oil, respectively [46]. Petrie et al. [47] describe the production of fish oil-like levels (>12%) of DHA in *C. sativa* seed oil and achieving a high ω3/ω6 ratio. The same T-DNA vector for producing fish oil-like levels of DHA in canola [27] was used to engineer DHA production in *B. juncea*, with up to 17% DHA produced in the T_4 seed oil of some *B. juncea* lines. Interestingly, some lines with a truncation of the T-DNA insert that eliminated the Δ4-desaturase activity stably accumulated 12% of ω3 DPA [48]. This was the first example of land plant-based oil seed ω3 DPA production, and was 2–3 times higher than most other natural sources. An exception for the occurrence of ω3 DPA is the abalone, a group of small to very large marine gastropod molluscs in the family Haliotidae, which can contain elevated ω3 DPA, e.g., 13–14% [49]. The distribution of abalone globally is restricted to a limited number of countries, with only a comparatively small harvest available. Abalone would not be able to serve as a sustainable large-scale source of ω3 DPA.

The development of a new and sustainable source of ω3 DPA further demonstrated the capability of engineering a complex metabolic pathway in different oilseed crops. This demonstration also offered a sustainable source of ω3 DPA, which is currently only available from wild oceanic species or seals with limited quantities for commercial use. Studies have also shown that DPA is much more effective at reducing the risk of cardiovascular disease, and that DPA is more effective than EPA at promoting endothelial migration [10]. A 1:1 ratio (*w*/*w*) of DHA to DPA in *B. juncea* seed oil has also been achieved [48]. The combination of DHA and DPA may be an excellent future dietary means for promoting cardiovascular health. Other attempts for producing stearidonic acid (SDA, 18:4ω3), a medium chain length ω3 PUFA, were also reported in linseed [50] and soybean [51]; the latter has been deregulated in the USA.

3. The Challenge of Deregulation

Development of oilseed crops with fish oil-like levels of ω3 LC-PUFA generates one of the most novel nutritional traits through genetic engineering, which is regulated in many production and import markets. Although the terms "genetic modification" or "genetically modified organism" are commonly used to refer to agricultural biotechnology or its products, USA regulatory authorities, such as the USDA and FDA, do not recommend using these terms because they are less accurate and could encompass the broader spectrum of genetic alterations, including some traditional and new breeding technologies [52]. In addition, according to the US National Bioengineered Food Disclosure Standard, the "bioengineered" distinction is now required for mandatory compliance in the United States [53]. In this review, the term "genetic engineering" has been used to describe the technology. Genetically engineered crops have established a history of safe use since their first recorded commercialization in the mid-1990's [54]. Since then, most countries that either produce or import biotechnology products have developed and implemented rigorous policies to regulate genetically engineered crops and their derived products. Although safety assessments should be science-based without geographic borders, the country- or regional-based regulatory systems have indeed created many challenges for approving and adopting biotechnology products on a broader scale and in a timely fashion. Different regulations and measurements about genetically engineered products also create complexities for the movement of agricultural commodities between countries and have a significant impact on international trade.

In addition to the challenges that are caused by various regulatory requirements in different jurisdictions, nutritionally enhanced products face unique challenges for deregulation because nutritional quality or traits are generally more complex and involve multiple genes to function. As of today, most approved and commericalized genetically engineered crops are focused on agronomically improved traits, such as herbicide tolerance or insect resistance. The introduction of a single gene to express the specific protein for the intended use makes it relatively easy to characterize the insertion structure, protein safety, and interactions with the environment. In fact, many of the regulatory data requirements for risk assessments are established based on these single-gene traits, such as determining the impact on non-targeted organisms and weediness potential. However, for nutritionally enhanced traits, the introduction of a multiple gene pathway generates new challenges for characterization. For example, a detailed molecular characterization is required for regulatory approval, and traditionally it has relied on Southern blot analysis to establish transgene locus and copy number along with targeted sequencing of PCR products spanning any inserted DNA. However, Southern blot analysis is labor intensive and time-consuming; therefore, it can be very expensive for characterizing complex events that contain multiple genes and/or multiple copies.

The use of sequencing-based technologies, such as whole-genome sequencing and junction sequence analysis bioinformatics, proves to be more efficient and effective at identifying and characterizing complex events and provides in-depth sequence-level information [55–57]. Therefore, for nutritional traits obtained with a multiple-gene pathway, next-generation sequencing is the preferred and recommended approach for molecular characterization. Further, unlike the insect resistance or herbicide tolerance traits, nutritional quality traits generally do not present plant pest properties; therefore, risk assessment should be trait-based. Some regulatory requirements for assessing impacts on non-target organisms or weediness potential might not be relevant or applicable to some nutritional traits.

The ω3 LC-PUFA are marine-sourced nutrients and the multiple-gene pathway for their biosynthesis that exists in algae is not native to higher plants, making genetic engineering the only viable tool to produce these valuable LC-PUFA in oilseed crops, such as canola. Currently, there are two new genetically engineered canola events that have been developed to produce ω3 LC-PUFA. One is the canola event NS-B5ØØ27-4, also known as DHA canola, described above. The other is the canola event LBFLFK from BASF. Both events have multiple genes introduced from yeast and microalgae to express various desaturases and elongases that are required to convert OA to ω3 LC-PUFA in canola seed.

One important aspect of a risk assessment is to evaluate the safety of newly introduced proteins in genetically engineered crops. The desaturases and elongases introduced in ω3 LC-PUFA oilseed crops are integral transmembrane proteins, which are intractable proteins, making them extremely difficult to isolate and purify for protein characterization and safety studies [58].

Another challenge of characterizing transmembrane proteins is to achieve a suitable level of recombinant protein expression and purity to successfully generate antibodies [59]. Moreover, these introduced desaturases and elongases share high levels of amino acid sequence homology between individual desaturases or elongases. This makes it difficult to quantify the individual expressed protein levels in the transgenic crops using conventional Western blot analysis. Western blot analysis is only possible if the antibody against the specific desaturase or elongase is available, as antibodies may suffer from cross-reactivity. A targeted LC-MS/MS method was developed to simultaneously quantify the seven biosynthetic pathway enzymes in DHA canola [60]. The targeted peptides for LC-MS/MS detection can be defined in silico for each specific protein. Even a single amino acid residue variation in a highly homologous region of two proteins can be detected separately with a highly selective and sensitive multiple reaction monitoring mass spectrometry assay. The absolute quantification of the targeted peptides was achieved by adding synthetic peptides identical in sequence but labelled with stable isotopes at the C-terminal arginine or lysine leading to a mass shift of +10 or +8 daltons. This approach confirmed that the enzymes that drive the production of DHA using seed-specific promoters were detected only in the seed of DHA canola at low levels [60]. A similar LC-MS/MS approach was applied for determining the in vitro protein digestibility of these transmembrane proteins, using simulated gastric conditions [61]. It has been shown that all the enzymes in the biosynthetic pathway were readily digested.

Desaturases and elongases are naturally found in a wide range of organisms with a long history of safe use. They are rapidly digestible and heat-instable, and no safety concerns were identified from the bioinformatics analysis and protein characterization [62]. Therefore, there is little value in conducing animal toxicity studies. Nevertheless, some jurisdictions require protein toxicity studies despite of the weight of evidence of their safety. Considering it is impractical to conduct such studies using purified proteins, alternative approaches using crude proteins or whole foods, such as meal and oil, should be accepted.

To address the different requirements for safety assessments in different jurisdictions, comprehensive safety evaluations for these new ω3 LC-PUFA canola events have been conducted using the weight of evidence approach. This included a detailed molecular characterization, a safety assessment of gene sources, a history of safe use, in silico bioinformatic analysis for allergenicity and toxicity potential, in vitro digestibility and stability studies, comparable agronomic field studies and compositional analysis, dietary exposure evaluations, toxicity testing, and animal feeding studies, amongst others. All the studies confirmed that these two ω3 LC-PUFA canola events (NS-B5ØØ27-4 and LBFLFK) were as substantially equivalent as conventional canola varieties; the only difference observed was the expected oil profile change [37,62]. Fish feeding trials also demonstrated that DHA canola oil [62–64] or *Camelina* EPA oil [65–68] were safe and effective alternatives to fish oil in the fish diet. Another study using ω3 LC-PUFA canola oil to replace menhaden fish oil in shrimp diets also showed no significant differences in shrimp performance metrics [69].

DHA canola has been approved for food, feed, and cultivation in Australia, Canada, and the USA. Given its unique total omega-3 profile for human nutrition and health, Nutriterra® Total Omega-3, the brand name of DHA canola oil for the human consumption market from Nuseed, was also recognized as a new dietary ingredient by the US FDA, allowing its use as a nutraceutical supplement in the USA. The canola event LBFLFK was also approved for cultivation, food, and feed use in Canada and the USA. Both ω3 LC-PUFA canola events are targeted to substitute fish oil in aquaculture feed. Most recently, Nuseed's Aquaterra® omega-3 canola oil was authorized for use in fish feed in Norway, making history as the first biotech product to receive approval in this market, the world's largest salmon producer.

It is also important to point out that, unlike input traits, nutritionally enhanced crops are typically produced under the identity preservation (IP) system to maintain their product identity and integrity. For example, Nuseed's DHA canola is produced under a closed-loop stewardship IP system to closely track and manage its ω3 LC-PUFA trait from seed to oil.

4. Market Demand and Consumer Acceptance for ω3 Canola Oil and Applications

The necessity for novel ω3 sources is driven by rapidly increasing demand and urgent environmental challenges. Fish oil is the historic primary source of ω3 LC-PUFA and is typically sourced through wild-caught fish. About 75% of this oil is directed to the aquaculture industry with the remainder going to human nutrition markets [70]. Looking closer at these multiple channels reveals the market readiness and opportunity for ω3 canola oils.

A key aspect for market consideration is consumer opinion. In the early stages of the CSIRO research, consumer preference trials were performed in Australia, the USA, Europe, and Asia [71–73]. When conducted up to 15 years ago, these studies concluded that a large proportion of the population are accepting of genetically engineered land plants producing omega-3 LC-PUFA oils that provided a health benefit, that were supported by health claims from a trusted source, and were indirectly consumed (e.g., food for farming fish).

5. Aquaculture

All feeds manufactured for use in salmonid aquaculture contain marine ingredients. Although marine ingredients are renewable resources, the supply cannot keep up with the rapid growth of aquaculture. The aquaculture industry has expanded in the past twenty years, and now delivers about 50% of all seafood for human consumption [74], but wild-caught fisheries have maintained overall static production levels since the late 1980s. This creates a shortage of raw marine materials and challenges the economic and environmental sustainability of fisheries and aquaculture [74].

The declining availability of marine-sourced oils against the increased demand has led to significant changes to fish feed formulations; specifically, higher incorporation of plant-derived and animal by-products, where approved, are replacing marine ingredients [16]. In Norway, the marine oil content of feeds declined from 24% in 1990 to 10.9% in 2013 [75]. Mowi's annual report for 2019 indicates their use of fish oil in salmon diets was 10% [76]. Mowi is also by far the world's largest Atlantic salmon farmer with harvest volumes of 466,000 tonnes in 2021, equivalent to a global market share of approximately 20% [77]. The Chilean average marine oil incorporation in fish feeds was 7% in 2017 [78].

The composition of dietary fatty acids affects many aspects of fish growth and health. According to Robert [79], nutritionally compromised diets often increase a species' susceptibility to infectious diseases. There is now growing concern about the effect that low availability of omega-3 fatty acids could have on the ability of fish to survive disease or environmental challenges.

Unsurprisingly, a decline in EPA and DHA in salmon feed formulations resulted in a reduced omega-3 content in salmon fillets [14,15], reducing the nutritional value of salmon for human consumption. Consumers are also increasingly concerned for the environmental impact of aquaculture, in particular the dependency on foraged fish to produce farmed fish. These are measured in the industry as the Fish In–Fish Out ratio (FIFO) and the Foraged Fish Dependency Ratio (FFDR), and salmon producers are under pressure to reduce these metrics without compromising omega-3 nutrition for fish welfare and human nutrition.

For all these reasons, aquaculture is enthusiastically embracing the innovation of ω3 canola oils. This uptake is represented in Figure 2, with the application of the DHA canola now occurring. Aquaterra® Advanced Omega-3, the DHA canola oil being marketed by Nuseed, contains significant levels of DHA and other ω3 PUFA and ω3 LC-PUFA (35% of total fatty acids). Oil harvested from 1–2 ha DHA canola contained the same amount of DHA that would be available from 10,000 kg of wild caught fish (Figure 2). The oil also contains high levels of ALA and has a much higher ω3/ω6 ratio in comparison with conventional canola oil [27,37,62]. The unique combination of high DHA, high ALA, and a high ω3/ω6 ratio are the major attributes of Aquaterra®, which led to impressive results in a commercial scale feeding trials and industry recognition [62,80].

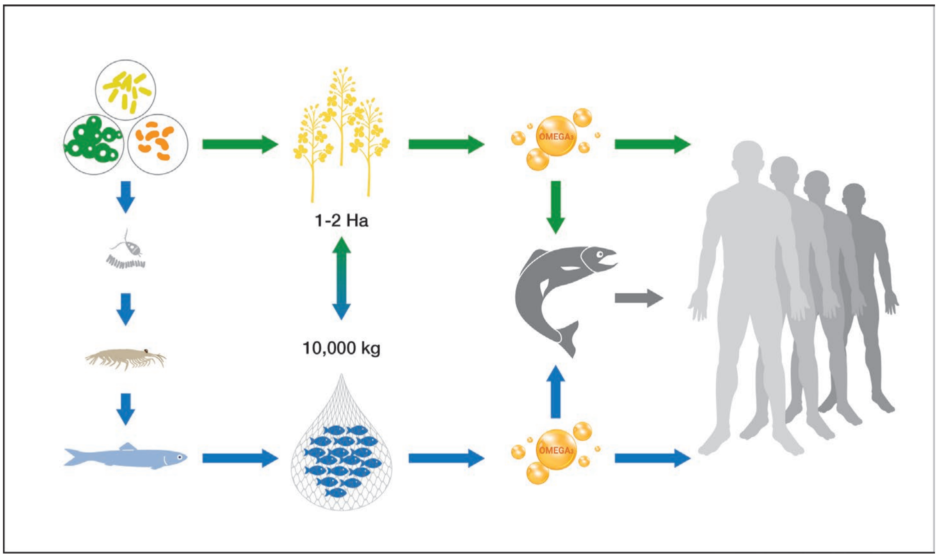

Figure 2. The combined sources of ω3 LC-PUFA are shown, with the traditional main sources (left and bottom, arrows in blue) being seafood and microalgae. New sources of ω3 LC-PUFA are now available through the availability of genetically engineered oilseed plants for use in both feed and food/supplement applications; these are indicated along the top row in green.

6. Human Nutrition

While most of the oil harvested from wild-caught fish is used in aquaculture, the global human nutrition market for ω3 LC-PUFA currently requires over 115,000 MT each year, and this is projected to grow at 2.1% annually [81]. The human nutrition market is experiencing the same supply constraints as the aquaculture industry, which leaves supplement manufacturers in need of sustainable alternative sources to meet current demand. There is also significant interest in plant-based omega-3 sources. A survey conducted from 1200 vitamin consumers discovered 64% would prefer a plant-based alternative, driven by a combination of organoleptic and environmental concerns [82]. These findings are in line with the CSIRO consumer research on oilseed-derived omega-3 sources noted earlier [71–73].

ω3 LC-PUFA containing oils from genetically engineered *Camelina* or canola have been confirmed as effective replacements for fish oil as a dietary source of ω3 LC-PUFA for mice, rats [83,84], and humans [85–88]. Nutriterra® is expeller-pressed from DHA canola seed, then refined, bleached, and deodorized. Like Aquaterra®, it delivers a unique omega-3 profile and a beneficial (high) ω3/ω6 ratio, with a milder sensory experience than occurs for marine-based ω3 LC-PUFA. The Nutriterra® product demonstrated bioavailability and efficacy in a human clinical trial [89]. Sixteen weeks of daily supplementation of the DHA canola oil was found to be safe and well-tolerated [89]. Additionally, Nutriterra® is certified by Friend of the Sea [90] to provide consumer confidence in the sustainability claims.

7. Conclusions and Perspective

Production of sustainably grown and harvested ω3 LC-PUFA canola events is now a commercial reality (Figure 2), and importantly, it can provide triple bottom-line benefits (economic, social, and environmental) for the world. The global market for the ω3 LC-PUFA oils containing EPA and DHA is estimated to be USD$3B. As demand for DHA and EPA increases, there are significant prospects for the global expansion of the ω3 LC-PUFA containing canola crops. For example, a DHA canola crop of just 1.25 M ha (~3.5% of global canola production) would be required to replace 50% of fish oil currently sold globally. It is further estimated that 2.5 M ha of the DHA canola crop can provide equivalent amounts of DHA to that currently harvested from all fish oils [91].

Crop-based production of ω3 LC-PUFA will enable increased consumption of omega-3 oils by consumers. A report [92] on the global burden of human disease estimated that there was a USD$31B cost in terms of productivity and 1.4 B premature deaths due to ischemic heart disease associated with low intake of DHA and EPA. The report estimates that with the use of dietary supplements of DHA and EPA, pre-term births could be significantly reduced, resulting in USD$6.1B in healthcare savings in the USA and USD$51M in Australia. These dietary supplements could also reduce heart disease in adults; this benefit is associated with an estimated USD$485M in savings.

In terms of environmental considerations, nearly 60% of wild-caught fish is used to provide omega-3 oil for the burgeoning global aquaculture industry. One widely cited paper describes the amount of large predatory fish in the oceans as being at only 10% of pre-industrial times [17]. One of the authors claimed in a later publication that the state of the oceans, combined with the impact of climate change and the effect of these factors on fish stocks, were such that 'all commercial fish and seafood may collapse' by 2048 [93]. A land-based sustainable and scalable source of the health-benefitting DHA and EPA (Figure 2) can play a critical role in providing nutritional security and reducing the chance of depleting wild fish stocks.

Author Contributions: Conceptualization, X.-R.Z., S.S.; writing, all. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: We thank the wider CSIRO and Nuseed Omega-3 team members for their contributions and financial support from CSIRO, GRDC, and Nuseed to the past research project. We also thank Megan Shaw for critical reading and editing on this review.

Conflicts of Interest: The authors declare no conflict of interest.

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