Modification of Plant Lipids for Human Health: Development of Functional Land-Based Omega-3 Fatty Acids

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ABSTRACT We have remodeled canola seeds to accumulate the omega-3 fatty acid, stearidonic acid (SDA). In doing so, we have demonstrated the feasibility of developing a land-based source of functional omega-3 fatty acids on a large scale. Land-based omega-3 fatty acids represent a sustainable source of omega-3 fatty acids that can be produced on large acreages and delivered to consumers in a wide variety of functional foods. And unlike α-linolenic acid, SDA can provide eicosapentaenoic acid equivalence at moderate intakes. Widely applied, SDA-enriched foods could become a valuable tool for delivering recommended levels of omega-3 fatty acids to large portions of the population. By obviating the need for dietary changes, SDA-enriched foods may facilitate increased compliance with recommendations for daily omega-3 intake.

KEY WORDS: • canola oil • stearidonic acid • PUFA • omega-6 fatty acids • omega-3 fatty acids • biotechnology

Background
Dietary fats and oils represent a significant percentage of the daily caloric intake in the United States comprising >33% of total calories (1). A large body of scientific evidence has implicated the quantity and/or quality of dietary fats in the development of several diseases, including cardiovascular disease (CVD),1 some cancers and arthritis (2). Not surprisingly, organizations including the USDA, American Heart Association and National Academy of Sciences/Institute of Medicine have made dietary recommendations recently that focus not only on the quantity but also on the types of fats in the diet, and generally recommend substituting monounsaturated and polyunsaturated fats for saturated fatty acids (SFA) (3). Because PUFA are not synthesized de novo in mammals, they must be derived from the diet. Once ingested, they are further metabolized and the resulting Long Chain PUFA populate cellular membranes and serve as precursors for hormone-like eicosanoids. Dietary PUFA are almost exclusively plant derived. In plants PUFA are derived from SFA. SFA are progressively desaturated to form monosaturated fatty acid, oleic acid (OA) [18:1(n-9)] and the PUFA, linoleic acid (LA) and α-linolenic acid (ALA) [18:2(n-6) and 18:3(n-3), respectively]. Depending on the position of the first double bond in the fatty acid molecule, polyunsaturates are classified as either omega-6 (n-6) or omega-3 (n-3) fatty acids (Fig. 1). The PUFA biosynthetic pathway occurs in virtually all plant cells, hence, omega-6 and omega-3 fatty acids are present in varying proportions in leaves, seeds and oil, and from there are incorporated into the diet. In the U.S. polyunsaturated fats constitute ~7% of total energy intake. LA compromises up to 89% of total PUFA energy intake, whereas ALA typically comprises only about 10% total PUFA energy in adult diets (4). This relative disparity in (n-3) to (n-6) intake is reflected in the (n-3) to (n-6) ratio of the most widely consumed vegetable oil in the U.S. diet, soybean oil, which constitutes ~83% of the vegetable oil intake (5) (Fig. 2).

Omega-3 fatty acids, known to be essential for growth and development, have been positively associated with health and the prevention and treatment of heart disease, arthritis, inflammatory and autoimmune diseases and cancer (2). Accordingly, there are now dietary recommendations and guidelines for omega-3 fatty acid intakes. For example, in a recent Scientific Statement, the AHA Dietary Guidelines suggest Americans consume at least two servings of fish per week, and include in the diet vegetable oils rich in the omega-3 fatty acid, ALA (6). It also recommends that 1.3–2.7 g/d total omega-3 fats be consumed. Despite these recommendations, it is estimated that actual dietary intakes of omega-3 fatty acids, and eicosapentaenoic (EPA) and docosahexaenoic (DHA) fatty acids are far below these recommended levels.
SFA; monosaturated fatty acids; PUFA, polyunsaturated fatty acids; ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; SDA, stearidonic acid.

There is consensus for the need for increased dietary omega-3 intakes among populations where intakes are below recommended levels. However, it remains unknown how to achieve this for large sectors of the population, especially where dietary preferences exclude fish. The concept of enriching a wide variety of foods with omega-3 fatty acids, so that consumers can choose omega-3 enriched foods that suit their individual preferences, is logical and has been proven in concept as an effective means to increase omega-3 intakes on a wide scale (7). Presently, sources of omega-3 fats suitable to enrich foods include vegetable and nut oils, which can contain up to 50% ALA, marine oils and EPA and DHA in varying amounts (4) and single-cell oils that are derived from the fermentation of microalgae, which contain DHA and/or EPA. However, there are limitations to each of these sources. The bioconversion of dietary ALA to EPA, which is necessary for the therapeutic and preventative benefits of omega-3 fats, is extremely inefficient with as little as 0.2% of plasma ALA undergoing conversion to EPA (8). Nonhydrogenated fish oils, on the other hand, provide EPA and DHA, the most effective forms of dietary omega-3 fatty acids for decreasing CVD risk and improving overall health. However, persistent questions exist about the sustainability of global fisheries. It is estimated that to achieve the recommended levels of EPA and DHA, a fourfold increase in fish consumption in the United States is necessary (4). Yet, yields from global fisheries have been reported to be stagnant or declining (9), and although aquaculture is a rapidly growing source of fish, the dietary requirements of omega-3 containing farmed fish for EPA and DHA requires fish meal and fish oil be provided in their diets. Hence, the same supply pressures that are facing wild stocks of fish will likely impact farmed fish (10). In addition to issues of sustainability, there is increasing alarm over levels of methyl mercury in some species of long-lived fish, that has prompted warnings to limit consumption of certain fish species, including swordfish, mackerel and shark, and for some at risk groups, avoid consumption all together (Environmental Protection Agency; National Academy of Sciences; Institutes of Medicine). Oil from fermentation of microalgae can be very high in EPA or DHA. Once extracted, it is suitable for enrichment of food, however, it is not currently being produced in sufficient quantities for wide-scale impact.

**Stearidonic acid**

We have previously proposed that the omega-3 fatty acid, stearidonic acid (SDA) [18:4(n-3)], a metabolic intermediate between ALA and EPA, could effectively bridge the gap between the sustainability of a land-based oil and the effectiveness of fish oil (11). SDA is an intermediate between ALA and EPA and can represent up to 4% of the total fatty acids in fish oil (12), and thus has been present in the human diet for as long as fish, such as menhaden, have been in the diet. SDA represents the Δ6 desaturation product of ALA and as such, bypasses a rate-limiting step in the conversion of dietary ALA to EPA. The relative effectiveness with which dietary SDA is bioconverted to EPA is thus dependant upon regulation of the subsequent desaturation and elongation reactions (Fig. 1). Recently, the relative efficiencies of dietary SDA, ALA and EPA in elevating plasma EPA was assessed in a clinical study (11). In this study, dietary SDA was found to increase plasma EPA between threefold and fourfold more efficiently than comparable levels of ALA and was approximately one-third as effective as dietary EPA (Fig. 3). At this relative rate of metabolic conversion of SDA to EPA, moderate intakes of SDA-containing oil could positively impact plasma EPA to an extent that would be expected to confer the cardiovascular benefits associated with consumption of EPA. Through incorporation into a variety of widely consumed foods, SDA could significantly increase omega-3 intakes to such an extent that the health benefits of a diet containing adequate omega-3 fats can be realized by a larger sector of the population.

Natural sources of SDA do exist, but are limited such that wide-scale enrichment of foods is currently impossible. In

![FIGURE 2 Fatty acid composition of the major vegetable oils](https://example.com/fatty-acid-graph.png)
addition to its occurrence in fish oil, SDA is also found in the seed oil and leaves of a number of plants, including evening primrose (Oenothera biennis), borage (Borago officinalis), black currant (Ribes nigrum) and Echium plantagineum. However, none of these species represent crops adapted to wide scale production and yields of oil are low and variable and not economically competitive. One approach to achieving SDA production on a large scale would be to implement an intensive breeding program for a species such as Echium plantagineum for adaptation to commercial scale agriculture, although the feasibility of this is questionable. An alternative approach, and one we have attempted successfully, is to engineer the capacity to produce SDA in to a high oil-yielding oilseed crop where existing commercial cropping and oil extraction technologies are utilized. That approach is presented below.

Genetic engineering of plant lipids

The genetic modification of oilseed crops provides an opportunity to tailor the composition of seed oils for optimal dietary or processing characteristics. Until recently, modifications of oil composition could be achieved through traditional plant breeding, where natural diversity within closely related species could be exploited, or through mutagenesis. Transgenic technology widens the scope of modifications achievable in oil composition by allowing the introduction of a wider range of genetic elements than is otherwise possible. There has been considerable focus on the identification and analysis of enzymes and underlying genes involved in plant lipid biosynthesis (13). Coupled with efficient plant transformation systems for the key oilseed crops, canola and soybean, metabolic engineering of seed lipids in these two crops has become feasible. As a result there has been successful development of a variety of novel oils in which the fatty acid composition has been optimized for improved processing characteristics (14), healthfulness (15) or industrial applications (16).

Development of SDA-canola

In conventional canola oil, ~97% of the lipids are represented in three classes: SFA (5.25%, 16:0 and 18:0), monounsaturated fatty acids (66%, 18:1) and PUFA (26%, 18:2 and 18:3) (Fig. 4), with the PUFA containing an ~2:1 ratio of LA to ALA. SDA, which is not normally present in canola oil, represents the Δ6 desaturation product of ALA. Because canola accumulates primarily OA in its oil, the production of significant quantities of SDA in canola required increased flux through the PUFA pathway from OA to ALA and then the addition of Δ6 desaturase activity (Fig. 1). Genes encoding the fatty acid desaturase enzymes that catalyze these reactions have been identified and characterized from diverse sources including higher plants and fungi (17). We generated transgenic canola lines that expressed in seeds the Δ6 and Δ12 fatty acid desaturases isolated from the commercially grown fungus, Mortierella alpina, and the Δ15 fatty acid desaturase from canola (Brassica napus). Seed oil from independent transformants accumulated SDA, as predicted (18). SDA accumulated up to 23% of the oil by weight, although the amount of SDA and the ratio of SDA to other seed lipids was influenced by the strategy utilized. For example, in one approach, the Δ6, Δ15 and Δ12 desaturases were combined on the same transformation vector. Resulting canola seeds were evaluated for seed fatty acid composition in the first transgenic generation and selected lines were self-pollinated and evaluated in the subsequent generation. The fatty acid composition of one line is shown in Figure 4. In this line, SDA accumulated to ~16% of the total fatty acids. The total omega-3 content in the seed lipids (ALA + SDA) was ~60% of the fatty acids whereas the total omega-6 fatty acid content of the seed lipids (GLA + LA) was ~22%. OA was reduced from 60% of the seed lipids to ~12%. In an alternative approach, a crossing strategy was utilized wherein transgenic lines containing only the M. alpina Δ6 and Δ12 desaturase genes were hybridized with independently produced lines containing only the B. napus Δ15 desaturase gene. Seed lipids were evaluated in the F1 and F2 generations from several independent crosses. In these progeny, SDA accumulated up to 23% of the lipids F1 seed (Fig. 4). The total omega-3 content in the seed lipids (ALA + SDA) exceeded 55% of the seed lipids whereas the total omega-6 fatty acid content of the seed lipids (GLA + LA) was ~22% of the seed lipids.

CONCLUSIONS

The enhancement of vegetable oil quality is an exciting and valuable application of biotechnology. We have produced prototype canola lines that have been engineered to accumu-
late in the seed oil, the omega-3 fatty acid, SDA. Oil from these lines would be suitable for incorporation into a range of foods, and by comparison to conventional canola oil and soybean oil, have significantly higher levels of effective long chain omega-3 fatty acid precursors and an improved omega-3 to omega-6 ratio (Table 1) Additionally, because SDA is approximately one-third as efficient as EPA, modest intakes of SDA-containing vegetable oil can be expected to provide the health benefits of recommended fish intakes. A wide variety of SDA-enriched foods, if available, could supply a significant portion of the recommended daily intakes of omega-3 fatty acids to large numbers of consumers. In addition, specific populations exist that could realize even greater benefit from the availability of SDA-enriched foods. These include vegetarians and vegans, whose omega-3 status may border on biochemical deficiency (19) and pregnant women who are faced with contradictory recommendations that include increasing omega-3 intake and decreasing intake of several species of fatty fish including shark, swordfish and king mackerel because of elevated levels of methyl mercury (20).

A large nutritional gap exists between recommended and actual daily intakes of omega-3 fatty acids (2) and it is widely accepted within the scientific and nutritional communities that this contributes to a variety of health consequences. If current advice to increase omega-3 intakes is heeded, demand for omega-3 fatty acids will increase as will the need for alternative sources to fish and fish oil. Biotechnology may provide a truly unique solution to the supply of dietary omega-3 fats by simultaneously solving the existing intrinsic problems of availability and sustainability, as well as that of bioactivity.

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**LITERATURE CITED**


**TABLE 1**

<table>
<thead>
<tr>
<th>Vegetable Oil Source</th>
<th>Total Omega-3 (% fatty acids)</th>
<th>n-6:n-3 ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sunflower</td>
<td>0</td>
<td>69:1</td>
</tr>
<tr>
<td>Corn</td>
<td>1</td>
<td>61:1</td>
</tr>
<tr>
<td>Soybean</td>
<td>7</td>
<td>7:1</td>
</tr>
<tr>
<td>Canola</td>
<td>12</td>
<td>2.6:1</td>
</tr>
<tr>
<td>SDA-Canola*</td>
<td>50</td>
<td>1.2</td>
</tr>
</tbody>
</table>

* Based on composition of F2 Seed, Reported in Knutson, 1999 (ref. 24). SDA, stearidonic acid.