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GENETIC ENGINEERING OF PLANT LIPIDS

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ABSTRACT

Vegetable oils are a major component of human diets, comprising as much as 25% of average caloric intake. Until recently, it was not possible to exert significant control over the chemical composition of vegetable oils derived from different plants. However, the advent of genetic engineering has provided novel opportunities to tailor the composition of plant-derived lipids so that they are optimized with respect to food functionality and human dietary needs. In order to exploit this new capability, it is essential for food scientists and nutritionists to define the lipid compositions that would be most desirable for various purposes.

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INTRODUCTION

Vegetable oils account for about 85% of the world's edible fat and oil production (Table 1); the remainder is derived from animal and marine sources. In 1994–1995 in the United States, the average daily intake of refined fats and oils was approximately 82 g per person (Table 2). Animal products—including red meats, poultry, fish and shellfish, milk and milk products, and eggs—may contribute more than half the total fat to American diets. The relationship between dietary fats and chronic disease in humans has long been recognized. Epidemiologic studies have consistently indicated a strong, continuous, and positive relationship between total cholesterol levels in blood and the prevalence and incidence of, and mortality from, atherosclerotic vascular disease, especially coronary heart disease. Studies of dietary lipids and blood pressure of humans also suggest that a diet with a high polyunsaturated-to-saturated ratio and low total fat can produce modest reductions in blood pressure in normotensive and hypertensive persons. Patterns of fat consumption associated with decreased risk of cardiovascular disease are also associated with a decreased risk of cancer and diabetes. Recent studies of human neonates have shown that the intake of long-chain polyunsaturated fatty acids (PUFAs) has beneficial effects on cardiovascular disease, renal function, and the development of normal function of the retina and brain.

Table 1 World production of major sources of fats and oils 1995–1996[a]

Commodity	World production[b]
Cottonseed	3.8
Olive	1.5
Peanut	3.8
Rapeseed	11.2
Soybean	19.7
Sunflower	8.9
Coconut	3.2
Palm	15.1
Palm kernel	2.1
Butter (fat content)	4.3
Tallow	8.0
Fish oils	1.3

[a] From Reference 96a.
[b] Millions of metric tons.

Table 2 Per capita usage of fats and oils in 1994–1995 by US consumers[a]

Commodity	Usage (lbs)
Butter	4.8
Lard and tallow	5.0
Margarine	9.9
Baking and frying fats	24.1
Salad and Cooking Oils	24.3
Other	1.6

[a]From Reference 96a. lbs, pounds.

Efforts to predict the impact of diverse fats and oils on degenerative disease risk are complicated by differences in fatty acid profile, fatty acid chain length, degree of saturation, positional isomerization of *cis* and *trans* unsaturated bonds, triglyceride structure, presence of lipid oxidation products such as oxysterols, and presence of antioxidants. The development through plant genetic engineering of functional or "designer" foods based on considerations of value to human health represents an opportunity to improve the health and well-being of a vast number of people worldwide.

This review summarizes recent developments in the directed genetic modification of higher plants to produce edible fats and oils with a predefined fatty acid composition and stability, and the relationship of these fats and oils to the prevention and control of chronic diseases. In 1997 in the United States, approximately 18 million acres of transgenic oilseeds were planted (23) and more is being planted all the time. Most of the plants released to date have been modified for traits not directly related to oil composition. However, modified oil composition is a high priority, and now that the first transgenic plants have been approved and well received by growers, it will not be long until plants with modified oil composition are commonplace. Because it typically requires more than five years to develop a commercially useful genetically engineered plant variety, some of the information presented here is prospective inasmuch as it is based on results obtained with model plants such as tobacco rather than with crop species. More detailed information about the basic plant biology related to oilseed engineering may be found in several recent reviews (12, 58, 72, 73, 96).

PLANT LIPID METABOLISM AND GENETICS

The Composition of Edible Oils

Because the lipid composition of plant membranes is thought to play a role in the ability of plants to survive exposure to cold temperatures or other stresses, there is a substantial body of work concerning the genetic modification of the lipid composition of plant tissues (73). However, because lipids account for only about 0.5% of the weight of leaves, fruits, roots, and other edible portions of most plants, except seeds and mesocarp tissues, the lipid composition of these tissues is a relatively minor component of human diet. Most of the plant lipids used for food are obtained from the seeds or mesocarp tissues of a small number of species generally well suited to efficient large-scale production (Table 1). The species used to produce mesocarp-derived lipids (e.g. palm and olive) are currently not amenable to genetic manipulation, and therefore all the genetic engineering work to date is focused on manipulating seed lipids of temperate field crops.

The predominant constituent of all refined edible vegetable oils is a mixture of triacylglycerols (TAGs), which may comprise as much as 60% of the dry weight of seeds from some plant species. In the cells of the seeds, TAGs are contained in specialized organelles, the lipid bodies, where they are surrounded by a phospholipid monolayer stabilized by hydrophobic proteins called oleosins (1). During germination of the seeds, the TAG is metabolized as a carbon and energy source to support the growth of the seedling until it can become photoautotrophic. Because the TAG does not play any structural role in membranes, wide variation in the fatty acid composition of TAG is observed in different plant species (97). The biological reason for variation in seed TAG composition among different species of plants is not known. In some cases it is believed that the fatty acid composition has been selected to make the seeds unpalatable or nonnutritional to certain pests and pathogens. Whatever the precise biological reason for the diversity of composition, it is clear that higher plants can store and metabolize a wide variety of different fatty acids. Thus, it is widely assumed that major changes made in the fatty acid composition of oilseeds by genetic engineering will not be incompatible with the normal growth and development of modified plants.

Besides TAG, a large number of other constituents are present to varying degrees in vegetable oils, depending on the status of the seed or fruit at the time of extraction. Most processed vegetable oils contain varying amounts of partial glycerides, mono- and diacylglycerols, and unesterified free fatty acids. It is generally assumed that these constituents are not present to the same degree in plant tissues and are a byproduct of processing. Thus, one opportunity for future modification of plants will be to reduce or eliminate hydrolytic and oxidative enzymes, such as lipases and lipoxygenases, that lead to formation of these undesirable lipid species during extraction and processing.

Seeds also contain small amounts of sterols, of which there are many types. Plants do not synthesize cholesterol. The most abundant sterols in most plant oils are 4-desmethyl sterols such as sitosterol (3). Sphingolipids and small quantities of tocopherols and tocotrienols are also present in the membranes of plants, but they represent a small fraction of the fat in vegetable oils. Vegetable oils also contain various pigments, typically a mixture of carotenoids and chlorophylls. Although these minor constituents may exert significant effects on the stability or nutritional quality of a particular type of oil, relatively little is known about what controls their abundance.

Synthesis of Plant Lipids

In order to understand what kinds of changes in plant lipid composition are feasible, an overview of the salient features of lipid biosynthesis in plants is helpful. Plants synthesize fatty acids from acetyl-coenzyme A (CoA) using a fatty acid synthase that is similar to that found in *Escherichia coli*. In most plants, the primary product of fatty acid synthesis is a fully saturated 16-carbon fatty acid (i.e. 16:0). However, some species accumulate shorter fatty acids (e.g. 8:0, 10:0, 12:0, 14:0) as a result of the action of thioesterases that cleave the growing fatty acid from the acyl carrier protein (ACP) and, thereby, prevent further elongation. Many genes for thioesterases have been cloned. These have been used to create transgenic canola plants that accumulate caprylic acid (8:0), capric acid (10:0), and lauric acid (12:0) rather than 16- and 18-carbon fatty acids (18, 98, 99).

The palmitoyl-ACP produced by fatty acid synthesis may be incorporated into TAG without further modification or elongated to stearate by a specific isozyme of β-keto-acyl-ACP synthase (KAS). The relative amounts of palmitate and stearate produced are thought to be controlled by the ratio of thioesterase and KASII activities (50). In some plants, 18-carbon fatty acids may be further elongated by poorly characterized enzymes that catalyze the addition of two to six additional carbons. Most plants contain a family of eight or more distinct desaturases that can introduce up to three double bonds into the fatty acid chain (92). The 16-carbon fatty acids that become incorporated into chloroplast membranes become polyunsaturated by the sequential action of a family of lipid-linked desaturases (92). However, in most species, palmitoyl-ACP is a poor substrate for the stearoyl-ACP desaturase that introduces the first double bond into stearate. Thus, the 16-carbon fatty acids destined for TAG synthesis are generally released from the plastid as free palmitic acid and are not further desaturated. For fatty acids with 18 carbons, the first double bond is usually a *cis* unsaturation between carbons 9 and 10 to produce oleic acid (18:1n-9). Diunsaturated fatty acids usually contain a second *cis* double bond between carbons 12 and 13, and triunsaturated fatty acids usually contain the third *cis* double bond between carbons 15 and 16. Certain plant species, such as borage, have additional desaturases that introduce double bonds at other positions. For instance, γ-linolenic acid (18:3n-6) is a direct precursor of animal PUFAs such as arachidonic acid, eicosapentaenoic acid, and docosa hexaenoic acid.

Fatty acid synthesis takes place in plant-specific organelles called plastids. However, TAG synthesis takes place on the endoplasmic reticulum (ER), so fatty acids must move from the plastid to the ER, where they are incorporated into phosphatidic acid, which is rapidly converted to diacylglycerol. The action of a diacylglycerol acyltransferase on diacylglycerol results in the synthesis of TAG. TAG is thought to accumulate within the ER membranes between the two monolayers to form inclusions bounded by a single leaflet of the ER membrane. Subsequent budding of the inclusions results in maturing lipid bodies that are stabilized by a surface layer of phospholipids and oleosins. The combined specificities of the various acyltransferases result in characteristic species of TAGs (10, 60, 73). In plants that produce unusual fatty acids in their seed oil, these specificities are thought to be essential for their incorporation at selected positions into TAG. Thus, for instance, rapeseed oil contains erucic acid at the sn-1 and sn-3 positions, but not at the sn-2 position.

Genetic Modification of Fatty Acid Composition

Until recently, limited modifications of the lipid composition of crop plants has been achieved through plant breeding techniques (see 48, 74, 87). Plant breeders have traditionally made use of the natural diversity that exists among plant varieties and closely related species to transfer desirable characteristics from one to another. Some examples of the range of variation in plant fatty acid composition within species are shown in Table 3. Thus, for example, the

Table 3 Fatty acid composition of some common edible oils[a]

	% composition				
Fatty acid	Cocoa butter[b]	Coconut	Corn	Olive	Soybean
6:0	0	<1.2	0	0	0
8:0	0	3.4–15	0	0	0
10:0	0	3.2–15	0	0	0
12:0	0	41–56	0	0	0
14:0	0.2–0.16	13–23	>0.1	0	<0.1
16:0	23.6–30.5	4.2–12	8.0–19	7–17.8	7.0–14
16:1	0.1–0.4	0	<0.5	0	<0.5
17:0	0.1–0.2	0	0	0–0.1	0
18:0	30.2–36.5	1.0–4.7	0.5–4.0	2.2–4	1.4–5.5
18:1	332.–38.6	3.4–12	19–50	43.7–78.2	19–30
18:2	2.2–4.8	0.9–3.7	34–62	5–32.3	44–62
18:3	∼0.3	0	<2.0	0–1.5	4–11
20:0	0.7–1.4	<0.1	<1.0	0	<1.0
20:1	0	0	0	0–0.5	<1.0
22:0	∼0.2	0	<0.5	0	<0.5
24:0	0	0	<0.5	0	0

[a] From Reference 28. Results shown as percentage composition.

[b] From *Theobroma cacao*.

oleate content of canola oil may vary between 43% and 78% of total fatty acids. The variations in composition within a plant species may be caused by varietal differences, by effects of growing conditions (e.g. temperature), or by simple genetic differences. For example, although rapeseed oil naturally contains high levels of erucic acid (22:1n-9), a single mutation in an elongase gene effectively abolished accumulation of the long-chain fatty acid (59). This mutation, which is the basis of the distinction between canola and rapeseed, inactivates a gene encoding a β-keto-acyl-ACP synthase that is specifically involved in elongation of 18-carbon fatty acids.

In cases where natural variation is limited, useful variation has been introduced by mutagenesis. Thus, for example, cultivars of sunflower in which as much as 90% of the fatty acids in TAG are oleate, and less than 7% saturates, have been recovered from mutagenesis-based breeding schemes (20, 22). Sunflower normally contains 66–72% linoleate, 11–12% saturates (palmitate and stearate), 16–20% oleate, and less than 1% alpha-linolenate. High-oleate sunflower is now in production and accounts for 10–15% of total production. Because of its stability, the oil does not require catalytic hydrogenation for stabilization.

Mutagenesis has also been used to produce a variety of flax that effectively eliminates linolenic acid and exhibits a corresponding increase in linoleic acid (47). By analogy with the rapeseed/canola distinction, the new flax cultivars based on the two mutations responsible for this effect are called linola, to distinguish them from traditional varieties that produce a drying oil for industrial uses. The corresponding oil is called solin. In soybean and rapeseed, mutagenized lines have been isolated with low seed oleate desaturase activity, resulting in the increased accumulation of oleic acid and decreased polyunsaturates in seed (6, 32, 78). The utility of the approach has been limited by the fact that many species are polyploid. Also, in some cases, the genes that control the fatty acid composition of storage lipids also affect the composition of membranes in other tissues. Thus, changes in the activity of these enzymes may have deleterious effects on plant vigor and productivity (67).

For most major oilseed crop species, methods for genetic transformation are now available. This opens up many opportunities to selectively modify their lipid composition and nutritional value by genetic engineering.

Availability of Genes

Directed genetic modification of plant lipids depends on the availability of genes for the enzymes that catalyze synthesis of many of the components of plant storage lipids. During the past several years, genes have been isolated for most of the relevant enzymes. Thus, representative genes are now available for the four different fatty acyl desaturases that introduce the double bonds required for synthesis of α- and γ-linolenate (25, 38, 52, 68, 89, 92). A gene encoding a $\Delta 5$ desaturase involved in the biosynthesis of eicosapentaenate was also recently isolated (65). In addition, a number of other desaturases have been identified that open up the possibility of producing fatty acid isomers that are not normally found to any significant extent in common sources of edible oils (14, 89). A broad range of thioesterases, which produce medium-chain fatty acids of varying lengths, have been cloned and characterized in transgenic plants (17, 98, 99). Genes for elongases that convert oleate to 20:1 and 22:1 have been cloned (39, 59). Also, the acyltransferases involved in synthesis of diacylglycerol have been cloned, and genes from different species exhibit different substrate specificities with respect to the range of fatty acids they will accept (10, 11, 53, 60). Thus, the basic genetic information required to control chain length, degree of unsaturation, and positional specificity is in hand. Work is continuing to characterize variants of this basic set of genes with different substrate specificities. However, in general, the identification of genes is no longer a major limitation.

Altered Enzymatic Activity Through Overexpression, Gene Silencing, and Antisense

Transgenic plants with increased level of activity for an enzyme of interest can usually be obtained by placing the corresponding gene under transcriptional control of a strong promoter. Directed methods for reduction of enzyme activity in plants have generally utilized gene silencing through antisense or cosuppression technologies. The antisense method involves the introduction of a modified gene that produces RNA transcripts complementary to the gene to be silenced. Various mechanisms for antisense suppression of gene expression have been proposed involving direct or indirect repressive effects on transcription and/or translation of the target gene mRNAs (9). The second method, termed cosuppression, involves the introduction into transgenic plants of all or a portion of the target coding sequence in the sense orientation under the control of a strong promoter. Most of the resulting transgenic plants exhibit high levels of expression of the transcript and the corresponding gene product. However, in most cases, a few percent of the transgenic plants exhibit strongly reduced or no expression of the introduced gene or the corresponding endogenous gene. The mechanisms underlying cosuppression are also unknown. One hypothesis is that the resulting down-regulation of target gene expression is a defensive reaction to the inflation of the mRNA pool above a critical threshold (45, 93). Cosuppression is usually observed only in a low proportion of primary transgenic events (about 1 in 100 transformants), but once a cosuppressed plant line is identified, the trait may be stably inherited. Cosuppression and antisense approaches both require that the transgene and the target gene share a minimum level of sequence similarity, usually more than about 70%. A major advantage of these methods, compared with mutagenesis, is that gene silencing can be restricted to a tissue of choice by placing the cosuppression or antisense construct under the transcriptional control of a tissue-specific promoter.

METABOLIC ENGINEERING TO IMPROVE THE NUTRITIONAL QUALITY OF PLANT LIPIDS

Reduction of Saturates

The principal saturated fatty acids in vegetable oils are palmitate and stearate. Because these two fatty acids are generally not equivalent with respect to dietary effects or food functionality, there is widespread interest in being able to control the relative amounts of these fatty acids. There has been progress in at least three complementary strategies for meeting this objective.

One goal is to regulate the relative amounts of stearate and palmitate. This is thought to be controlled by the ratio of palmitoyl thioesterase and KASII activities (50). Thus, Kinney (50) obtained a small but significant decrease in palmitate and a corresponding increase in the amount of stearate/oleate by forcing high levels of expression of a gene for KASII in soybean and tobacco. Conversely, by decreasing the amount of soybean KASII by cosuppression, a significant increase in the amount of palmitate was obtained at the expense of 18-carbon fatty acids (50). Increasing the amount of thioesterase activity by overexpressing an acyl-ACP thioesterase (FATB) in plants where KASII was cosuppressed resulted in soybean oil containing up to 50% palmitate (51).

A theoretical strategy for reducing palmitate and stearate levels in some oils is to enhance the extent of unsaturation by introducing genes for novel desaturases. There are now many examples in which heterologous genes from plant, animal, yeast, and bacterial origins encode a functional protein when expressed in transgenic plants (12, 38). Examples of the use of this technology in the field of plant lipid engineering are the transfer into transgenic tobacco of the genes encoding the yeast and animal stearoyl-CoA desaturases (26, 76). The significance of these experiments was that in most plants, the plastid stearoyl-ACP desaturase is the only enzyme capable of converting stearate to oleate. Thus, the ratio of 18:0 to $18{:}1 + 18{:}2 + 18{:}3$ in TAG is thought to be regulated by competition between the plastid enzymes stearoyl-ACP thioesterase and stearoyl-ACP desaturase. Once stearate is hydrolyzed from ACP and exported from the plastid, it is not normally a substrate for desaturation. By contrast, in yeast and mammals, stearate is desaturated in the ER by stearoyl-CoA desaturase. Thus, by expressing the yeast or animal stearoyl-CoA desaturase in plants, it is possible to provide a second opportunity to desaturate stearate that has reached the

ER. A second potentially useful effect of expressing stearoyl-CoA desaturases in plants was that the level of palmitoleate (16:1n-9) increased and the level of palmitate decreased. This is due to the fact, noted elsewhere, that palmitate is a poor substrate for plant stearoyl-ACP desaturase but is a good substrate for stearoyl-CoA desaturase. This may be useful for decreasing the level of palmitate in edible oils.

Petroselinic acid (18:1n-12) is an isomer of oleic acid with a *cis* double bond between carbons 6 and 7. By contrast with oleic acid, which is a liquid at room temperature, petroselinic acid is solid at room temperature, with a melting point of 33°C. Because of this property, and because of the positive health effects of monounsaturated fatty acids (MUFAs), petroselinic acid has been considered for the manufacture of solid fats. Species such as carrot and coriander have relatively high levels of petroselenic in their seed lipids because of the action of a $\Delta 4$ palmitoyl-CoA desaturase and a subsequent elongation step (13, 14). Expression of the corresponding gene resulted in the accumulation in transgenic tobacco of very small amounts of the unusual fatty acid, which suggests that it may eventually be possible to produce commercial quantities of this fatty acid (14).

When fed to laboratory rats, petroselinic acid, although readily absorbed, has (*a*) led to significantly greater liver weights (81, 100) and (*b*) reduced the concentrations of arachidonic acid in tissue lipids by inhibiting the desaturation and chain elongation reactions to linoleic acid (100, 101). Thus, although petroselenic has good physical properties with respect to food functionality, it may not be a suitable dietary component.

Decreasing the Oxidation Potential of Plant Fatty Acids

Kubow (56) has noted that PUFAs and plant sterols, when exposed to oxidative stress either thermally or through aeration, may form potentially toxic oxidized products that can have powerful atherogenic effects (62, 75). Because frying subjects vegetable oils to lengthy heat treatments, and light-induced oxidation prevents their long-term storage, a major objective of oil crop engineering is to decrease the PUFA content in oil crops such as soybean and rapeseed to improve oxidative stability and enhance shelf life of the oil.

Linoleic acid (18:2n-6), because of its low-density lipoprotein (LDL) lowering effect, has been the main PUFA used in test diets (55). Although metabolic and epidemiological studies of humans and laboratory animals generally support the concept that higher intake of PUFAs are beneficial in terms of lipoprotein metabolism and cardiovascular health (30), the consumption of high amounts of PUFA when insufficiently protected by antioxidants has led to enhanced susceptibility of membrane lipids to peroxidation (95). Many studies have shown that dietary supplementation with either n-6 or n-3 PUFA is associated with increased susceptibility of tissue lipids to peroxidation both in vivo and in vitro.

In view of all the negative effects of lipid oxidation, vegetable oils with reduced levels of PUFAs and a higher content in MUFAs have become highly desirable. Nutritionally, oleic acid (18:1) appears to have the same LDL lowering effect as linoleic acid and is not as susceptible to in vivo oxidation as linoleic acid (55). A higher ratio of oleic to linoleic acid is inversely correlated with the peroxidation rate of plasma LDL. Because of its low cost, soybean oil is widely used for frying. Thus, researchers have sought to increase the ratio of MUFAs to PUFAs in soybean and canola oil by modifying the activity of a microsomal membrane-bound oleate desaturase designated FAD2.

Canola plants that express an oleate desaturase antisense construct under control of a seed-specific promoter have 18:1 levels above 80% in seed TAG and are currently in field trials. When the antisense construct was used in a line of canola that carried a mutation that reduced the activity of oleate desaturase, the oleate levels were above 88% and there was also a 2% decrease in saturates (32). Similarly, varieties of soybean accumulating oils that are rich in oleic acid have been produced through antisense or cosuppression of the seed oleate desaturase (32). Whereas normal soybeans have about 20% 18:1 and 15% saturates, the cosuppression lines contained more than 80% 18:1 and ~11% saturates. An antisense construct based on the linoleate desaturase also reduced linolenate levels in soybean TAG from about 8% to less than 2% (32). As expected, the high oleate fatty acid composition conferred much-improved heat stability and shelf life over the traditional product. Thus, for instance, whereas conventional soybean oil has an AOM rating (active oxygen method for fat stability) of 10–20 h, the best high-oleic transgenic lines have AOM ratings of up to 140 h (23). Similar experiments were conducted with canola. In both cases, the silencing of FAD2 provided a substantial reduction of the problems of in vitro and in vivo oxidation of seed oil (16). Approximately 80,000 acres of the transgenic high-oleic soybeans were grown in 1998 as a trial, and it is expected that the modified plants will become a major new commodity.

Eliminating Trans *Fatty Acids*

An ongoing trend has been to substitute solid fats derived from plant oils for animal fats. Industrial processes have been developed to partially hydrogenate vegetable oils to form semisolid or solid fats. Although hydrogenated vegetable oil has been the best nutritional alternative to saturated animal fats, this process results in the formation of undesirable *trans* isomers of unsaturated fatty acids. The oils with a high proportion of *trans* isomers tend to have unfavorable effects on plasma concentrations of total cholesterol, triglyceride, LDL, and high-density lipoproteins (46, 55, 64, 104, 105). The average dietary uptake of *trans* fatty acids in western countries is estimated at 7–8 g per person per day, of which about 80% are contributed by hydrogenated oils (22). A promising approach to reducing *trans* fatty acids is to eliminate the need for hydrogenation. For some applications, this can be accomplished by using very high–oleic sunflower oil. For example, several modified sunflower varieties contains 87–90% oleic acid and only about 6.5% saturates.

A related objective of researchers working on modifying plant lipids has been to obtain transgenic line–producing oils with a high stearate and low palmitate content; these oils do not require extensive hydrogenation. Nutritionally, palmitate increases plasma LDL cholesterol. Stearate, on the other hand, appears to have a neutral effect on total plasma cholesterol and LDL cholesterol (27, 55, 85). High stearate content has been achieved in canola through antisense reduction of the expression of stearoyl-CoA desaturase (23, 54). One major drawback of increasing the stearate content of oilseeds is that it may have deleterious effects on seed development and germination (67). As a result, it may not be possible to completely eliminate the use of hydrogenation in the production of solid fats from plant oils derived from plants grown in temperate regions.

Production in Plants of Essential Long-Chain PUFA

Members of the n-3 and n-6 PUFA (α-linolenic acid and linoleic acid) are important constituents of cell membranes (29) and serve as precursors for long-chain PUFAs with carbon lengths of C20/C22, which subsequently form a variety of biologically active compounds known as eicosanoids (e.g. prostaglandins, leukotrienes, and thromboxanes). These eicosanoids play a critical role in coordinating physiological interactions among cells and influence a wide variety of functions, including those of the central nervous system and the contractions of smooth muscles. They also inhibit the mobilization of fatty acids from adipose tissue, have an antiinflammatory effect, and influence blood pressure, the aggregation of blood platelets, and cardiac function. A potential need for dietary long-chain PUFAs (20:4n-6 and 22:6n-3) in some groups, particularly newborn infants, is a major area of current research (41). Supplementation with long-chain PUFAs in formula foods produces beneficial effects on cardiovascular disease, renal function, and the development of normal function of the retina and brain (2, 37, 60a, 103). The consumption of these long-chain PUFAs (C20/C22) also has beneficial effects in patients with chronic inflammatory diseases (88), a history of colonic polyps (34), ulcerative colitis (4, 84, 94), and genetic disorders of peroxisomal biogenesis (5, 83), such as Zellweger syndrome. One alternative to high intakes of long-chain n-3 fatty acids to control chronic inflammatory diseases such as rheumatoid arthritis is thought to be dietary supplementation with γ-linolenic acid (C18:3n-6) derived from the oils of evening primrose and borage plants (42, 66).

Genes encoding $\Delta 6$ linoleate desaturases have been cloned from a variety of organisms (25, 68, 71, 80, 86). Expression of borage and *Synechocystis* genes in transgenic tobacco resulted in the accumulation in leaves of substantial amounts of γ-linolenic and octadecatetraenoic acids (80, 86), a first step toward the production of γ-linolenic acid, eicosapentaenoic acid, and arachidonic acid in crop plants.

Production of Structured Lipids

Common vegetable oils and fats predominantly consist of TAGs with carbon numbers ranging from 12 to 22. Pancreatic lipase acting on dietary TAGs specifically hydrolyzes the fatty acids at the sn-1 and sn-3 positions, resulting in free fatty acids and 2-monoacylglycerols. Free fatty acids of medium and short chain lengths are absorbed into intestinal epithelial cells with high efficiency (57). Similarly, all 2-monoacylglycerols are well absorbed, irrespective of the chain length at the sn-2 position of the glycerol molecule. However, long-chain free fatty acids (C20 and greater) are not absorbed as efficiently as are medium- and short-chain fatty acids, and much of the long-chain fatty acids may be lost as calcium–fatty acid soap in the feces. Hence, there is interest in developing sources of TAGs with selected fatty acids esterified in the sn-2 position.

Recently, structured TAGs (STAGs), which combine advantages from conventional fats with those used for special purposes, have become available by chemically catalyzed interesterification (24). During interesterification, the fatty acids randomly interchange position on the glycerol backbones of the TAG pool (79). In this way, it is possible to modify the melting characteristics of fats or mixtures of fats without changing the fatty acid composition of the blend. These STAGs have unique chemical, physical, or physiologic properties that are not observed by simply blending mixtures of the starting fats and oils. Although most STAGs are currently used for medical applications, some appear in confectionery products (24). Most currently available structured lipids are “medium-chain triglycerides” (MCTs), which are manufactured from fractionated caprylic acid (8:0) and capric acid (10:0) from such oils as coconut and palm kernel. However, because MCTs do not contain essential fatty acids, they are used with long-chain triglycerides containing essential fatty acids (e.g. linoleic, linolenic, or arachidonic acids). There is some evidence that structured lipids containing medium-chain fatty acids and linolenic acid may be more useful than mixtures of MCTs and long-chain triglycerides for treating lipid malabsorbtion syndromes (35).

In contrast to chemically catalyzed interesterification, enzyme-catalyzed interesterification can produce lipids of predefined structure (79). Although enzyme-catalyzed interesterfication has been used to produce research quantities of lipids, the cost has been a hurdle that has discouraged commercialization of targeted structured lipids. An alternative to the use of interesterification is to engineer plants to produce desirable structured lipids. In principle, this requires only that plants be engineered to produce the correct ratios of desired fatty acids and to contain acyltransferases that will produce the desired TAG structure. As a first step toward this goal, genes for a family of thioesterases have been cloned and characterized. For instance, the California bay is a tree that produces medium-chain fatty acids in seeds as a result of the presence of an acyl-ACP thioesterase (FATB) with high affinity for medium-chain fatty acids (15, 77, 99). Transgenic rapeseed plants expressing the gene accumulated up to 65% lauric acid in their seed TAG (98). These transgenic lines have been commercialized by Calgene: In 1997 in the United States, approximately 70,000 acres were planted for commercial production (23, 24). Because the sn-2 acyltransferase from canola has a high degree of specificity for an unsaturated fatty acid, the TAG produced by these plants contains predominantly 12:0/18:n/12:0 at the sn-1, sn-2, and sn-3 positions, respectively. The oil is being used to produce confectionery coatings, coffee whiteners, whipped toppings, and filling fats.

Recently, other thioesterase genes have been isolated from species of the genus *Cuphea*, which encode enzymes specific for C8 and C10 fatty acids. Transgenic rapeseed plants expressing these genes also accumulated significant amounts of these medium-chain fatty acids (17). Thus, it seems likely that it will soon be possible to produce a variety of MCTs in transgenic canola and other species.

An important recent result related to the engineering of plants to produce medium-chain fatty acids was the isolation of genes encoding lysophosphatidic acid sn-2 acyltransferases (10, 11, 53, 60). Attempts to isolate these genes were prompted by the observation that medium-chain fatty acids are systematically excluded from the sn-2 position of TAG in transgenic rapeseed (98). This was caused by the discrimination of the rapeseed acyltransferase against the unusual fatty acids. Expression of selected lysophosphatidic acid acyltransferases in transgenic plants should make possible engineering of plant oils to contain nutritionally useful structured TAG molecules. The fatty acid in the sn-2 position of TAG is preferentially absorbed as the 2-monoglyceride and serves as the template for reesterification by intestinal cells to reform triglycerides. The sn-2 fatty acids are also preferentially preserved as components of chylomicrons and very-low-density lipoprotein particles for ultimate incorporation in tissue membranes (57). Technology is evolving to create STAGs with a selected fatty acid in this sn-2 position. For instance, lipids that incorporate linoleic, arachidonic, or eicosapentaenoic acid at the sn-2 position are being evaluated for the specific objective of modulating serum cholesterol concentrations and essential fatty acid requirements (8). Use of medium-chain fatty acids (C8/C10) in STAGs is also of interest for patients with deficiencies in fat absorption, for critically ill patients dependent on total parenteral nutrition (40), or for use in infant formulas. Whether medium-chain fatty acids increase serum cholesterol levels is unknown (55).

Increasing Antioxidants

It is widely accepted that the basic function of vitamin E in animal tissue is to trap peroxyl free radicals and prevent propagation of the oxidation of unsaturated fatty acids. Dietary vitamin E applies to a family of eight related tocopherol and tocotrienol compounds. The four major forms of vitamin E are α-, β-, δ-, and γ-tocopherol. α-Tocopherol appears to be the most bioactive of all forms; however, its absorption is relatively inefficient, ranging from 20% to 80%, with an apparent half-life in plasma of 48 h.

The recommended daily allowance of vitamin E is 8–10 mg/day, and oral doses of up to 80–100 mg/day appear to be well tolerated (69). Larger daily intakes of vitamin E, although relatively nontoxic, may interfere with the absorption of vitamins A and K (91). The presence of large amounts of PUFAs in the diet may increase the need for α-tocopherol. In addition, increasing the amount of vitamin E in the diet has beneficial health effects, such as reducing the risk of cardiovascular disease and boosting immune function.

There is an interest in engineering crops such as soybean to accumulate increased amounts of α-tocopherol, given that soybean oil, which represents 80% of the oil consumed in the United States, is relatively poor in α-tocopherol despite its high content in total tocopherols. Recently, the gene encoding a methyltransferase that converts γ- to α-tocopherol was isolated from *Arabidopsis*. Seeds of transgenic plants overexpressing this gene accumulated up to 80-fold more α-tocopherol than did control plants, without reduction in the total tocopherol amount (90).

Tocotrienols are compounds with vitamin E activity and are the most important natural antioxidants in fats and oils. Although tocotrienols are less widely distributed than tocopherols, they are present in sufficient amounts (∼500–750 ppm) in palm oil, including natural palm oil, refined palm oil, and commercial fractions of palm oil. The interest in tocotrienols is largely due to their relationship to cholesterol biosynthesis. Recent studies have indicated that tocotrienols inhibit the activity of 3-hydroxy-3-methylglutaryl CoA-reductase—the rate-limiting enzyme in cholesterol biosynthesis (33, 43, 49).

In addition to tocotrienols, plant sterols also lower plasma cholesterol levels. Phytosterols, in contrast to cholesterol, are poorly absorbed in the intestine and quickly secreted from the liver. Their effect is to lower cholesterol absorption by displacing it from micelles. There is now a debate on whether saturated phytosterols such as sitostanol are more efficient at lowering plasma cholesterol levels than are unsaturated sterols such as sitosterol or campesterol (44, 61, 102). Natural sitostanols exist as minor constituents of certain seed oils. One current source is corn fiber oil, a by-product of wet milling, where sitostanols are present as ferrulic acid esters. Sitostanols can also be derived from phytosterols by hydrogenation. Margarines currently marketed in Finland contain sitostanol-esters that are derived from tall oil (a byproduct of paper pulping). Because hypercholesterolemia affects an estimated 90 million adults in the United States, cholesterol-lowering phytosterols represent a potentially sizeable market (31). Although a number of genes encoding enzymes of the plant sterol biosynthesis pathway have been identified (7), major increases in the accumulation of phytosterols in transgenic plants are unlikely to result from the overexpression of such genes. It seems likely that the identification of regulatory proteins controlling this pathway will give the most productive leads toward engineering plants to accumulate higher levels of phytosterols.

CONCLUDING REMARKS

Great progress has been made during the past several years in identifying the plant genes that regulate TAG composition. The first commercial products based on the use of these genes to engineer plant lipid composition are already on the market, and several more varieties are in large-scale field trials. Although several important deficiencies in our understanding of plant lipid metabolism remain (73), the results of many genetic engineering experiments have been consistent with expectations. Thus, the prospects are that we will be able to tailor the oil composition of the temperate-zone field crops to meet predefined compositions. In addition, there is now substantial research interest in defining the genes for oil-related antioxidants with a view to engineering the composition of these constituents.

Because of the long lag time and large investments required to produce and test genetically engineered field crops (19, 48), it is essential that food scientists and nutritionists establish well-defined goals for lipid composition. Many of the traditional descriptions of plant oils by origin, or by such gross measures as the ratio of saturates/unsaturates, are not sufficiently precise to guide the rational design of engineered plants. There also appears to be a relative lack of knowledge about the dietary effects of some fatty acids, such as 16:1n-9 or 18:1n-12, which may be possible to produce in large quantities. Thus, it is to be hoped that future dietary studies will be formulated with a view to facilitating the eventual optimization of the composition of plant oils for human consumption by genetic engineering.

Literature Cited

1. Abell BM, Holbrook LA, Abenes M, Murphy DJ, Hills MJ, et al. 1997. Role of the proline knot motif in oleosin endoplasmic reticulum topology and oil body targeting. *Plant Cell* 9:1481–93
2. Adesuyi S, Cockrell D, Gamache D. 1985. Lipoxygenase metabolism of arachidonic acid in brain. *J. Neurochem.* 45:770–76
3. Åppelqvist LA. 1989. The chemical nature of vegetable oils. In *Oil Crops of the World*, ed. G Robbelen, RK Downey, A Ashri, pp. 22–37. New York: McGraw-Hill
4. Aslan A, Triadafilopoulos G. 1992. Fish oil fatty acid supplementation in active ulcerative colitis: a double-blind, placebo-controlled, crossover study. *Am. J. Gastroenterol.* 87:432–37
5. Aubourg P, Adamsbaum C, Lavallard-Rousseau M-C, Rocchiccioli F, Cartier N, et al. 1993. A two year trial of oleic and erucic acids (“Lorenzo’s Oil”) as treatment for adrenomyeloneuropathy. *N. Engl. J. Med.* 329:745–52
6. Auld DL, Heikkinen MK, Erickson DA, Sernyk JL, Romero JE. 1992. Rapeseed mutants with reduced levels of polyunsaturated fatty acids and increased levels of oleic acid. *Crop. Sci.* 32:657–62
7. Bach TJ, Benveniste P. 1997. Cloning of cDNAs or genes encoding enzymes of sterol biosynthesis from plants and other eukaryotes: heterologous expression and complementation analysis of mutations for functional characterization. *Prog. Lipid Res.* 36:197–226
8. Bell S, Bradley D, Forse R, Bistrian B. 1997. The new dietary fats in health and disease. *J. Am. Diet. Assoc.* 97:280–86
9. Bourque JE. 1995. Antisense strategies for genetic manipulations in plants. *Plant Sci.* 105:125–49
10. Brough CL, Coventry JM, Christie WW, Kroon JTM, Brown AP, et al. 1996. Towards the genetic engineering of triacyglycerols of defined fatty acid composition: major changes in erucic acid content at the sn-2 position affected by the introduction of a 1-acyl-sn-glycerol-3-phosphate acyltransferase from *Limnanthes douglasii* into oilseed rape. *Mol. Breed.* 2:133–42
11. Brown AP, Coleman J, Tommey AM, Watson MD, Slabas AR. 1994. Isolation and characterization of a maize cDNA that complements a 1-acyl sn-glycerol-3-phosphate acyltransferase mutant of *Escherichia coli* and encodes a protein which has similarities to other acyltransferases. *Plant Mol. Biol.* 26:211–23
12. Budziszewski GJ, Croft KPC, Hildebrand DF. 1996. Uses of biotechnology in modifying plant lipids. *Lipids* 31:557–69
13. Cahoon EB, Ohlrogge JB. 1994. Metabolic evidence for the involvement of a delta-4-palmitoyl-acyl carrier protein desaturase in petroselinic acid synthesis in coriander endosperm and transgenic tobacco cells. *Plant Physiol.* 104:827–37
14. Cahoon EB, Shanklin J, Ohlrogge JB. 1992. Expression of a coriander desaturase results in petroselinic acid production in transgenic tobacco. *Proc. Natl. Acad. Sci. USA* 89:11184–88
15. Davies HM, Anderson L, Fan C, Hawkins DJ. 1991. Developmental induction, purification, and further characterization of 12:0-ACP thioesterase from immature cotyledons of *Umbellularia californica*. *Arch. Biochem. Biophys.* 290:37–45
16. Debonte LR, Fan Z, Loh WH-T. 1997. *Seeds, Plants and Oils with Altered Fatty Acid Profiles*. Wayzata, MN: Cargill
17. Dehesh K, Jones A, Knutzon DS, Voelker TA. 1996. Production of high levels of 8:0 and 10:0 fatty acids in transgenic canola by overexpression of chfatb2, a thioesterase cDNA from *Cuphea hookeriana*. *Plant J.* 9:167–72
18. Eccleston VS, Cranmer AM, Voelker TA, Ohlrogge JB. 1996. Medium chain fatty acid biosynthesis and utilization in *Brassica napus* plants expressing lauroyl-ACP thioesterase. *Planta* 198:46–53
19. Food and Drug Administration. 1992. Statement of policy: foods derived from new plant varieties. *Fed. Reg.* 57:22984–3005
20. Fick GN. 1989. Sunflower. In *Oil Crops of the World*, ed. G Robbelen, RK Downey, A Ashri, pp. 301–18. New York: McGraw-Hill
21. Fitch Haumann B. 1994. Questions remain over hydrogenated fats. *Inform* 5: 358–63
22. Fitch Haumann B. 1994. Modified oil

may be key to sunflowers future. *Inform* 5:1198–210

23. Fitch Haumann B. 1997. Bioegineered oilseed acreage escalating. *Inform* 8:804–11
24. Fitch Haumann B. 1997. Structured lipids allow fat tailoring. *Inform* 8:1004–11
25. Girke T, Schmidt H, Zaehringer U, Reski R, Heinz E. 1998. Identification of a novel Δ-6-acyl-group desaturase by targeted gene disruption in *Physcomitrella patens*. *Plant J.* 15:39–48
26. Grayburn WS, Hildebrand DF. 1995. Progeny analysis of tobacco that express a mammalian Δ-9 desaturase. *J. Am. Oil Chem. Soc.* 72:317–21
27. Grundy S. 1994. Dietary fat. See Ref. 104, pp. 44–57
28. Gunstone FD, Harwood JL, Padley FD. 1986. *The Lipid Handbook*. London: Chapman & Hall
29. Hansen H. 1994. New biological and clinical roles for the n-6 and n-3 fatty acids. *Nutr. Rev.* 52:162–67
30. Harris W. 1997. n-3 Fatty acids and serum lipoproteins: human studies. *Am. J. Nutr.* 65(Suppl.):S1645–54S
31. Hicks P. 1998. Cholesterol-lowering plant oil products. *Gen. Engin. News* 18:6
32. Hitz WD, Yadav NS, Reiter RS, Mauvais CJ, Kinney AJ. 1995. Reducing polyunsaturation in oils of transgenic canola and soybean. In *Plant Lipid Metabolism*, ed. JC Kader, P Mazliak, pp. 506–8. London: Kluwer
33. Hood RL. 1995. Tocotrienols and cholesterol metabolism. See Ref. 74a, pp. 96–104
34. Huang Y-C, Jessup M, Forse RA, Flickner S, Pleskow D, et al. 1996. N-3 fatty acids decrease colonic epithelial cell proliferation in high-risk bowel mucosa. *Lipids* 31(Suppl.):S313–17
35. Ikeda I, Tomari Y, Sugano M, Watanabe S, Nagata J. 1991. Lymphatic absorption of structured glycerolipids containing medium-chain fatty acids and linoleic acid, and their effect on cholesterol absorption in rats. *Lipids* 26:369–73
36. Deleted in proof
37. Inns S. 1994. Essential dietary lipids. See Ref. 104, pp. 58–66
38. Ishizaki-Nishizawa O, Fujii T, Azuma M, Sekiguchi K, Murata N, et al. 1996. Low-temperature resistance of higher plants is significantly enhanced by a nonspecific cyanobacterial desaturase. *Nat. Biotechnol.* 14:1003–6
39. James DW Jr, Lim E, Keller J, Plooy I, Ralston E, et al. 1995. Directed tagging of the Arabidopsis FATTY ACID ELONGATION1 (FAE1) gene with the maize transposon Activator. *Plant Cell* 7:309–19
40. Jeevanandam M, Holaday N, Voss T, Buier R, Patersen S. 1995. Efficacy of a mixture of medium-chain triglyceride (75%) and long-chain (25%) fat emulsions in the nutritional management of multiple-trauma patients. *Nutrition* 11:275–84
41. Jimenez J, Boza J, Suarez MD, Gil A. 1997. The effect of a formula supplemented with n-3 and n-6 long-chain polyunsaturated fatty acids on plasma phospholipid, liver microsomal, retinal, and brain fatty acid composition in neonatal piglets. *J. Nutr. Biochem.* 8:217–23
42. Johnson M, Swan DD, Surette ME, Stegner J, Chilton T, et al. 1997. Dietary supplementation with linoleic acid alters fatty acid content and eicosanoid production in healthy humans. *J. Nutr.* 127:1435–44
43. Jones PJH. 1997. Regulation of cholesterol biosynthesis by diet in humans. *Am. J. Clin. Nutr.* 66:438–46
44. Jones PJH, MacDougall DE, Ntanios F, Vanstone CA. 1997. Dietary phytosterols as cholesterol-lowering agents in humans. *Can. J. Physiol. Pharmacol.* 75:217–27
45. Jorgensen RA. 1995. Cosuppression, flower color patterns, and metastable gene expression states. *Science* 268:686–91
46. Katan M, Zock P, Mensink R. 1995. *Trans* fatty acids and their effects on lipoproteins in humans. *Annu. Rev. Nutr.* 15:473–93
47. Keller WA. 1995. Sources of oilseeds with specific fatty acid profiles. See Ref. 77a, pp. 87–97
48. Kessler DA, Taylor MR, Maryanski JH. 1992. The safety of foods developed by biotechnology. *Science* 256:1747–49
49. Khor HT, Chieng DY, Ong KK. 1995. Tocotrienols: a dose dependent inhibitor for HMGCoA reductase. See Ref. 74a, pp. 87–96
50. Kinney AJ. 1996. β-Ketoacyl-ACP synthetase II genes from plants. *US Patent 5,500,361*
51. Kinney AJ, Knowlton S, Cahoon EB, Hitz WD. 1999. Re-engineering oilseed crops to produce industrially useful fatty acids. In *13th Int. Symp. Plant Lipids, Seville, Spain*. In press
52. Knutzon D, Huang YS, Thurmond J, Chan G, Chaudhary S, et al. 1998. Cloning and expression of *Mortierella alpina* desaturase genes in yeast and in plants. In *13th Int. Symp. Plant Lipids, Seville, Spain*. In press

53. Knutzon DS, Lardizabal KD, Nelsen JS, Bleibaum JL, Davies HM, et al. 1995. Cloning of a coconut endosperm cDNA encoding a 1-acyl-sn-glycerol-3-phosphate acyltransferase that accepts medium-chain-length substrates. *Plant Physiol.* 109:999–1006
54. Knutzon DS, Thompson GA, Radke SE, Johnson WB, Knauf VC, et al. 1992. Modification of Brassica seed oil by antisense expression of a stearoyl-acyl carrier protein desaturase gene. *Proc. Natl. Acad. Sci. USA* 89:2624–28
55. Kris-Etherton PM, Yu S. 1997. Individual fatty acid effects on plasma lipids and lipoproteins: human studies. *Am. J. Clin. Nutr.* 65 (Suppl.):162–44S
56. Kubow S. 1993. Lipid oxidation products in food and atherogenesis. *Nutr. Rev.* 51:33–40
57. Kubow S. 1996. The influence of positional distribution of fatty acids in native, interesterified and structure-specific lipids on lipoprotein metabolism and atherogenesis. *J. Nutr. Biochem.* 7:530–41
58. Lassner M. 1997. Transgenic oilseed crops: a transition from basic research to product development. *Lipid Technol.* 9:5–9
59. Lassner MW, Lardizabal K, Metz JG. 1996. A jojoba beta-ketoacyl-CoA synthase cDNA complements the canola fatty acid elongation mutation in transgenic plants. *Plant Cell* 8:281–92
60. Lassner MW, Levering CK, Davies HM, Knutzon DS. 1995. Lysophosphatidic acid acyltransferase from meadowfoam mediates insertion of erucic acid at the sn-2 position of triacylglycerol in transgenic rapeseed oil. *Plant Physiol.* 109: 1389–94

60a. Lien EL. 1994. The role of fatty acid composition and positional distribution in fat absorbtion in infants. *J. Pediatr.* 125:562–68

61. Ling WH, Jones PJH. 1995. Minireview: dietary phytosterols: a review of metabolism, benefits and side effects. *Life Sci.* 57:195–206
62. Maerker G. 1987. Cholesterol autoxidation—current status. *J. Am. Oil Chem. Soc.* 64:388–92
63. Deleted in proof
64. Mensink R, Katan M. 1990. Effect of dietary *trans* fatty acids on high-density and low-density lipoprotein cholesterol levels in healthy subjects. *N. Engl. J. Med.* 323:439–45
65. Michaelson LV, Lazarus CM, Griffiths G, Napier JA, Stobart AK. 1998. Isolation of a Δ-5-fatty acid desaturase gene from *Mortierella alpina*. *J. Biol. Chem.* 273:19055–59
66. Miller C, Ziboh V. 1988. γ-linolenic acid-enriched diet alters cutaneous eicosanoids. *Biochem. Biophys. Res. Commun.* 154:967–74
67. Miquel MF, Browse JA. 1994. High-oleate oilseeds fail to develop at low temperature. *Plant Physiol.* 106:421–27
68. Napier JA, Hey SJ, Lacey DJ, Shewry PR. 1998. Identification of a *Caenorhabditis elegans* Δ-6-fatty-acid-desaturase by heterologous expression in *Saccharomyces cerevisiae*. *Biochem. J.* 330:611–14
69. National Research Council. 1989. *Recommended Dietary Allowances*. Washington, DC: Natl. Acad. Sci. 283 pp. 10th ed.
70. Deleted in proof
71. Nunberg AN, Beremand P, Thomas TL. 1996. Cloning and expression of a delta-6-desaturase from *Borago officianalis*. *Plant Physiol.* 111(Suppl.):132
72. Ohlrogge JB. 1994. Design of new plant products: engineering of fatty acid metabolism. *Plant Physiol.* 104:821–26
73. Ohlrogge J, Browse J. 1995. Lipid biosynthesis. *Plant Cell* 7:957–70
74. Ohlrogge J, Browse J, Somerville CR. 1991. The genetics of plant lipids. *Biochim. Biophys. Acta* 1082:1–26

74a. Ong ASH, Niki E, Packer L, eds. 1995. *Nutrition, Lipids, Health, and Disease*. Champaign, IL: Am. Oil Chem. Soc.

75. Piche LA, Draper HH, Cote PD. 1988. Malondialdehyde excretion by subjects consuming cod liver oil vs. a concentration of n-3 fatty acids. *Lipids* 23:370–71
76. Polashock JJ, Chin CK, Martin CE. 1992. Expression of the yeast Δ9 fatty acid desaturase in *Nicotiana tabacum*. *Plant Physiol.* 100:894–901
77. Pollard MR, Anderson L, Fan C, Hawkins DJ, Davies HM. 1991. A specific acyl-ACP thioesterase implicated in medium-chain fatty acid production in immature cotyledons of *Umbellularia californica*. *Arch. Biochem. Biophys.* 284:306–12

77a. Przybylaski R, McDonald BF, ed. 1995. *Development and Processing of Vegetable Oils for Human Nutrition*. Champaign, IL: Am. Oil Chem. Soc.

78. Rahman SM, Takagi Y, Kubota K, Miyamoto K, Kawakita T. 1994. High oleic acid mutant in soybean induced by x-ray irradiation. *Biosci. Biotechnol. Biochem.* 58:1070–72
79. Rammamurthi S, McCurdy AR. 1995.

Interesterification—current status and future prospects. See Ref. 77a, pp. 62–86

80. Reddy AS, Thomas TL. 1996. Expression of a cyanobacterial δ-6-desaturase gene results in gamma-linolenic acid production in transgenic plants. *Nat. Biotechnol.* 14:639–42
81. Richter K, Mukherjee K, Weber N. 1996. Fat infiltration in liver of rats induced by different dietary plant oils: high oleic-, medium oleic- and high petroselinic acid oils. *Z. Ernahrungswiss.* 35:241–48
82. Deleted in proof
83. Roels F, Fischer S, Kissling W. 1993. Polyunsaturated fatty acids in peroxisomal disorders: a hypothesis and a proposal for treatment. *J. Neurol. Neurosurg. Psychiatry* 56:937
84. Ross E. 1993. The role of marine fish oils in the treatment of ulcerative colitis. *Nutr. Rev.* 52:47–49
85. Salter A, White D. 1996. Effects of dietary fat on cholesterol metabolism: regulation of plasma LDL concentrations. *Nutr. Res. Rev.* 9:241–57
86. Sayanova O, Smith MA, Lapinskas P, Stobart AK, Dobson G, et al. 1997. Expression of a borage desaturase cDNA containing an N-terminal cytochrome b_5 domain results in the accumulation of high levels of Δ-6-desaturated fatty acids in transgenic tobacco. *Proc. Natl. Acad. Sci. USA* 94:4211–16
87. Scarth R. 1995. Production of oilseeds with modified fatty acid composition. See Ref. 77a, pp. 97–104
88. Shahar E, Folson AR, Melnick SL, Tockman MS, Comstock GW, et al. 1994. Dietary n-3 polyunsaturated fatty acids and smoking-related chronic obstructive pulmonary disease. *N. Engl. J. Med.* 331:228–33
89. Shanklin J, Cahoon EB. 1998. Desaturation and related modifications of fatty acids. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 49:611–41
90. Shintani D, DellaPenna D. 1998. Elevating the vitamin E content of plants through metabolic engineering. *Science* 282:2098-100
91. Sokol RJ. 1994. Vitamin E. See Ref. 104, pp. 133–34
92. Somerville CR, Browse J. 1996. Dissecting desaturation: Plants prove advantageous. *Trends Cell Biol.* 6:148–53
93. Stam M, Mol JNM, Kooster JM. 1997. The silence of genes in transgenic plants. *Ann. Bot.* 79:3–12
94. Stenson W, Cort D, Rodgers J, Burakoff R, Deschryver-Kecskemeti K, et al. 1992. Dietary supplementation with fish oil in ulcerative colitis. *Ann. Int. Med.* 116:609–4
95. Suarez A, Faus M, Gil A. 1996. Dietary supplementation with long chain polyunsaturated fatty acids increases susceptibility of weanling rat tissue lipids to in vitro lipid peroxidation. *J. Nutr. Biochem.* 7:252–60
96. Toepfer R, Martini N, Schell J. 1995. Modification of plant lipid synthesis. *Science* 268:681–86
96a. US Dep. Agric. 1996. *Agricultural Statistics, 1995–1996.* http:// www.usda.gov/nass/pubs/agstats.html
97. van de Loo F, Fox B, Somerville CR. 1993. Unusual fatty acids. In *Plant Lipids*, ed. T Moore, pp. 91–126. Boca Raton: CRC
98. Voelker TA, Hayes TR, Cranmer AM, Turner JC, Davies HM. 1996. Genetic engineering of a quantitative trait: metabolic and genetic parameters influencing the accumulation of laurate in rapeseed. *Plant J.* 9:229–41
99. Voelker TA, Worrell AC, Anderson L, Bleibaum J, Fan C, et al. 1992. Fatty acid biosynthesis redirected to medium chains in transgenic oilseed plants. *Science* 257:72–74
100. Weber N, Richter K, Schulte E, Mukherjee K. 1995. Petroselinic acid from dietary triacylglycerols reduces the concentration of arachidonic acid in tissue lipids of rats. *J. Nutr.* 125:1563–68
101. Weber N, Vosmann K, Bruhl L, Mukherjee K. 1997. Metabolism of dietary petroselinic acid: a dead-end metabolite of desaturation/chain elongation reactions. *Nutr. Res.* 17:89–97
102. Weidmann T, Pates R, Beach JM, Salmon A, Brown MF. 1988. Lipid-protein interactions mediate the photochemical function of rhodopsin. *Biochemistry* 27:64–69
103. Weststrate JA, Meijer GW. 1998. Plant sterol-enriched margarines and reduction of plasma total and LDL-cholesterol concentrations in normocholesterolaemic and mildly hypercholesterolaemic subjects. *Eur. J. Clin. Nutr.* 52:334–43
104. Ziegler E, Filer LJ, eds. 1994. *Present Knowledge in Nutrition*. Washington, DC: Int. Life Sci. Inst.
105. Zock P, Katan M. 1992. Hydrogenation alternatives: effects of trans fatty acids and stearic acid versus linoleic acid on serum lipids and lipoproteins in humans. *J. Lipid Res.* 33:399–410