Review

High-Oleic and High-Stearic Cottonseed Oils: Nutritionally Improved Cooking Oils Developed Using Gene Silencing

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Key words: high-oleic, high-stearic, cottonseed oil, *trans* fatty acids

Gene technology and plant breeding are combining to provide powerful means for modifying the composition of oilseeds to improve their nutritional value and provide the functional properties required for various food oil applications. Major alterations in the proportions of individual fatty acids have been achieved in a range of oilseeds using conventional selection, induced mutation and, more recently, post-transcriptional gene silencing (PTGS). In particular, a number of high-oleic oils have been developed in order to provide high-stability cooking oils. These oils provide the opportunity to replace the current widespread use of saturated fats and hydrogenated oils that contribute significantly to increased risk of cardiovascular disease due to the effect of saturated and *trans*-fatty acids on elevating LDL cholesterol in the bloodstream. Similarly, oils with increased stearic acid content are being developed to enable the production of solid fats without the need for hydrogenation. We have recently applied hpRNA-mediated PTGS in cotton to down-regulate key fatty acid desaturase genes and develop nutritionally-improved high-oleic (HO) and high-stearic (HS) cottonseed oils (CSOs). Silencing of the *ghFAD2-1* Δ12-desaturase gene raised oleic acid content from 13% to 78% and silencing of the *ghSAD-1* Δ9-desaturase gene substantially increased stearic acid from the normal level of 2% to as high as 40%. Additionally, palmitic acid was significantly lowered from 26% to 15% in both HO and HS lines. Intercrossing the HS and HO lines resulted in a wide range of unique intermediate combinations of palmitic, stearic, oleic and linoleic contents. The oxidative stability, flavor characteristics and physical properties of these novel CSOs are currently being evaluated by food technologists.

Key teaching points:

- Traditional plant breeding approaches have produced some important alterations in oilseed fatty acid compositions. However, some oilseed species lack the required natural genetic variation or are not readily amenable to mutation breeding due to their complex genomic structure.
- Gene technology has provided plant breeders with powerful new tools for manipulating the composition of plant products.
- Post-transcriptional gene silencing (PTGS) has been developed to enable the expression of genes to be precisely down-regulated during oil synthesis in the developing seed.
- Gene silencing techniques are being used to alter the relative proportions of the major fatty acids present in cottonseed oil for the purpose of improving nutritional value without compromising functionality.

NUTRITIONAL IMPACT OF DIETARY FATS AND OILS

Fats and oils account for a substantial portion of the calorific value of the human diet, being ingested in their natural form as components of whole foods (e.g., meats, nuts) or in their extracted form either as ingredients in processed foods or as cooking mediums, salad oils and spreads. Per capita fats and oils consumption varies widely throughout the world and increases with greater affluence, to the point where in many western countries it is in excess of the 30% maximum value generally recommended by health authorities [1]. Consequently

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Journal of the American College of Nutrition, Vol. 21, No. 3, 205S–211S (2002)
Published by the American College of Nutrition

there has been considerable attention given in recent decades to the nutritional impacts of various fats and oils, in particular the influence of the constituents of fats and oils on cardiovascular disease, cancer and various inflammatory conditions.

An important early focus was the nutritional impact of the cholesterol found in animal fats, such as tallow, lard and milk-fat derived products. Dietary intake of cholesterol was demonstrated to increase significantly the levels of total cholesterol in the bloodstream, contributing to increased occurrence of atherosclerosis and consequently greater risk of cardiovascular disease [2]. However, it was also revealed that the fatty acids that comprise the fats and oils can themselves have significant effects on serum cholesterol levels. Nutritional research became more closely focused on the two different classes of serum cholesterol, the beneficial high-density lipoprotein form (HDL) associated with the removal of cholesterol from the bloodstream, and the undesirable low-density lipoprotein form (LDL) responsible for the movement of cholesterol within the bloodstream. High levels of LDL cholesterol were shown to be associated with increased risk of atherosclerosis and cardiovascular disease [3,4]. Furthermore, it became apparent that individual fatty acids in the diet can have opposite effects on the relative levels of LDL-cholesterol and HDL-cholesterol in the bloodstream and that they can play a greater role than actual dietary cholesterol intake in this regard (Fig 1). Initially it was considered that all saturated fatty acids and, in particular, myristic acid (C14:0) and palmitic acid (C16:0), the principal saturate present in the plant oils, had the undesirable property of raising serum LDL-cholesterol levels [5,6]. However, it then became well established that stearic acid (C18:0), the other main saturate present in plant oils, does not raise LDL-cholesterol like other saturates and may actually lower total cholesterol [7,8]. Stearic acid is therefore generally considered to be at least neutral with respect to risk of cardiovascular disease. On the other hand, unsaturated fatty acids, such as the monounsaturate oleic acid (C18:1) and the polyunsaturates linoleic acid (C18:2) and α-linolenic acid (C18:3), have the beneficial property of lowering LDL-cholesterol [5], thus reducing the risk of cardiovascular disease.

The growing consumer recognition that high levels of both cholesterol and saturated fatty acids in the diet can increase the risk of cardiovascular disease has been the primary driving force behind the recent reduction in consumption of animal fats, particularly in Western countries. This move away from animal fats has been facilitated by the ready availability of predominantly unsaturated, cholesterol-free vegetable oils from a wide range of oilseed crops (e.g., soybean, sunflower, rapeseed, cottonseed, peanuts). Most of these vegetable oils have levels of oleic, linoleic and linolenic acids that significantly exceed that of palmitic acid, and nutritionists now generally recommend that preference is given to such oils in the human diet. In fact, health authorities such as the American Heart Association and the Australian National Heart Foundation are now recommending that polyunsaturated fatty acid consumption should be increased to compose around 10% of total energy intake and that α-linolenic acid consumption should be around 2g per day.

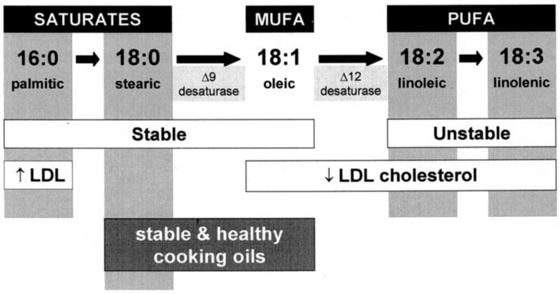

Fig. 1. Schematic diagram of biosynthetic pathway for the major saturated, monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids in oilseeds and their key nutritional and functional attributes.

Hydrogenated Oils

Unfortunately, although having beneficial nutritional effects, highly unsaturated oils are too unstable for use in cooking, particularly for commercial deep-frying where they are exposed to high temperatures and oxidative conditions for long periods of time. Under such conditions, the oxidative breakdown of the numerous carbon double bonds present in unsaturated oils results in the development of short-chain aldehyde, hydroperoxide and keto derivatives, imparting undesirable flavors and reducing the frying performance of the oil by raising the total level of polar compounds [9,10]. Furthermore, some of the breakdown products present in thermoxidized fats and oils are readily absorbed into the bloodstream and have been reported to have undesirable nutritional effects, including impaired arterial endothelial function and accelerated atherogenesis [11,12]. The beneficial effects of polyunsaturated fatty acids must therefore be delivered by other dietary means, such as in whole foods or in processed foods that are not subjected to highly oxidative conditions, such as spreads and salad oils.

Polyunsaturated oils can however be converted into stable cooking oils by the industrial process of hydrogenation in which the carbon double bonds (unsaturated) are reduced to single bonds (saturated) by the action of hydrogen in the presence of a catalyst [13]. Varying degrees of hydrogenation can be used depending on the initial content of polyunsaturated fatty acids and the desired final levels for particular product applications. Complete hydrogenation results in the elimination of all carbon double bonds and the production of a fully saturated fat. Partial hydrogenation enables oxidative stability to be achieved while retaining the liquid nature of the oil. Partially hydrogenated soybean, cottonseed and canola oils have been extensively used as frying oils in recent decades and have been one of the main ways in which the food service sector has increasingly replaced animal fats with stable vegetable oils. However, partial hydrogenation results in the breakdown of naturally occurring *cis* carbon double bonds and their occasional reformation in *trans* configuration [13,14], forming *trans*-fatty acids. In contrast to *cis*-unsaturated fatty acids, *trans*-fatty acids are now known to be as potent as palmitic acid in raising plasma LDL cholesterol levels [15,16] and lowering plasma HDL cholesterol [17], and thus contribute to increased risk of cardiovascular disease [18]. Although *trans*-fatty acids occur naturally in some other foods, particularly dairy products, average dietary intake is closely related to the use of hydrogenated oils and therefore varies considerably between countries [19]. For example, the average dietary intake of *trans*-fatty acids in the United Kingdom has been estimated at 2.8 grams per person per day, of which about 65% was considered to be contributed by hydrogenated oils [20]. As a result of increased awareness of the anti-nutritional effects of *trans*-fatty acids, there is now a growing trend away from the use of hydrogenated oils in the food industry in favor of fats and oils that are both nutritionally beneficial and can provide the required functionality without hydrogenation, in particular those that are rich in either oleic acid where liquid oils are required or stearic acid where a solid or semi-solid fat is preferred (Fig 1).

IMPROVED SEED OILS THROUGH PLANT BREEDING

Unfortunately, oils that comprise predominantly either oleic acid or stearic acid and that are also low in palmitic acid are not naturally common among the major cultivated oilseed species. However plant breeders have now successfully developed such desirable fatty acid profiles in a number of oilseed crops. In some oilseed species there has been sufficient natural genetic variability for fatty acid composition to enable the selection of high-oleic fatty acid profiles using conventional crossbreeding methods. The selection of high-oleic safflower [21] and peanut [22] oils from species that are normally high in linoleic acid are notable examples of this approach (Table 1). In other cases, where there has been insufficient natural variation, it has sometimes been possible to generate the required variability using induced mutagenesis techniques (Table 1). Mutation breeding was employed in sunflower to raise oleic acid from 29% to 84% [23], creating a high-oleic oil that has since become extensively used in both commercial and domestic frying applications [24]. Likewise, soybean mutants have been produced in which palmitic acid levels were reduced from 11% to 4% [25] and stearic acid raised from 4% to 30% [26]. High-stearic mutants of sunflower have also been produced [27]. In spite of these notable successes, in some oilseeds it has not been possible to develop the required fatty acid compositions using traditional plant breeding approaches alone because the species has lacked the required natural genetic variation and also has not been readily amenable to mutation breeding due to its complex genomic structure. However, newly developed gene technologies now provide the opportunity to overcome these limitations and enable radical alterations in fatty acid composition to be attempted in all major oilseed crop species.

IMPROVED SEED OILS USING GENE SILENCING

In recent years, genes have been cloned for all the major enzymes that control fatty acid biosynthesis in oilseeds, including the Δ9- and Δ12-desaturases that determine the relative proportions of C18 saturated, monounsaturated and polyunsaturated fatty acids (Fig. 1). Furthermore, methods of post-transcriptional gene silencing (PTGS) have been developed that

Table 1. Fatty Acid Composition of Traditional Oilseeds and Genetic Variants Derived from them by Selection, Mutation or Post-Transcriptional Gene Silencing (PTGS)

Variants[#]	Origin of Variant	Fatty acid composition (%)*				
		Palmitic	Stearic	Oleic	Linoleic	Linolenic
Safflower		7	6	10	77	—
high-oleic	selection	7	4	81	8	—
Peanut		8	4	55	25	—
high-oleic	selection	7	3	76	4	—
Sunflower		7	4	29	60	—
high-oleic	mutation	7	4	84	5	—
high-stearic	mutation	5	26	14	55	—
Soybean		11	4	24	53	7
high stearic	mutation	8	30	21	35	6
low palmitic	mutation	4	3	25	58	8
high-oleic	PTGS	8	3	84	3	1
Canola		4	2	57	26	9
high-oleic	PTGS	4	2	89	2	3
high-stearic	PTGS	4	33	13	19	22
Mustard		5	3	46	33	13
high-oleic	PTGS	3	3	76	7	6

[#] For each oilseed the first fatty acid profile is the traditional type.

* Minor fatty acids not reported; sources of data cited in text.

enable the expression of these genes to be precisely down-regulated during oil synthesis in the developing seed, without affecting their expression in other parts of the plant. PTGS can be invoked to modify seed oil fatty acid composition by seed-specifically expressing a DNA sequence that is complementary to the whole or part of the appropriate target fatty acid biosynthesis gene. The introduced DNA sequence is inserted into the genome in such a way that its transcription leads to the formation of a double-stranded RNA molecule (dsRNA), the presence of which triggers an inbuilt mechanism that degrades this dsRNA molecule and also the complementary mRNA transcripts of the endogenous target gene. The degradation of the target gene mRNA prevents the synthesis of its corresponding protein [28], in this case a fatty acid biosynthesis enzyme, thereby altering the balance of fatty acids present in the oils.

The earliest examples of PTGS involved the re-introduction of the full coding region of the target gene in either the normal (sense) or reverse (antisense) orientation. For example, antisense-mediated PTGS was used in rapeseed to down-regulate the expression of the Δ9-desaturase enzyme that converts stearic acid to oleic acid, resulting in an increase in stearic acid from 2% to around 33% [29]. Similarly, sense-mediated PTGS (co-suppression) targeted against the Δ12-desaturase that converts oleic acid to linoleic acid has resulted in the development of soybean, rapeseed (*Brassica napus*) and mustard (*B. juncea*) oils with very high levels of oleic acid [30,31]. However, these antisense and co-suppression strategies have proven to be variable and unpredictable in their effectiveness and generally require the production of large populations of transgenic plants in order to obtain an acceptable number of lines exhibiting sufficient degrees of target gene suppression [29,30,32].

Recently, much more effective methods of silencing plant genes have been developed, based on the discovery that PTGS can be invoked at very high frequency using inverted-repeat DNA constructs. Regardless of how they are inserted into the genome, these constructs always generate hairpin RNA (hpRNA) transcripts containing regions of dsRNA; therefore, a very high proportion of transgenic plants show target gene silencing [33]. The very high efficiency of hpRNA-mediated gene silencing makes it now the preferred method for tissue-specific gene inactivation in plants. We outline below our recent use of these techniques to develop high-oleic and high-stearic cottonseed oils [34], which represents the first application of hpRNA-mediated gene silencing to modify oil composition in plants and provides a good example of the advantages of this approach over other methods of PTGS in developing nutritionally enhanced cooking oils.

High-Oleic and High-Stearic Cottonseed Oils

Because of the large global volume of cotton crops grown primarily for fiber production, cottonseed is available in substantial quantities in many parts of the world. From the crushing of this seed, around four million tons of CSO are produced annually, making it the sixth most important plant oil in commerce. Cottonseed oil is a valued raw material in the food industry because its high level of the saturated palmitic acid and absence of the unstable linolenic acid (Table 2) impart good stability and flavor properties. However CSO is often partially hydrogenated to lower the level of polyunsaturates and achieve the very high stability required in deep-frying or the solidity required for margarine hard stock. Thus, partially hydrogenated CSO contains a relatively high level of nutritionally undesirable saturated and *trans* fatty acids. Genetic improvement of CSO fatty acid composition is therefore being sought to avoid the need for hydrogenation and thereby to improve the nutritional value of CSO products. Unfortunately, cotton has very limited genetic variation for seed fatty acid composition and is also not very amenable to induced mutation techniques. Furthermore, genetic transformation of cotton is much less efficient than in many other oilseeds. To overcome these limitations, we have recently taken advantage of the very high efficiency of hpRNA-mediated gene silencing to produce novel CSOs rich in either oleic acid or stearic acid and which also have significantly reduced levels of palmitic acid [34].

The genetic modifications were achieved by seed-specific silencing of the two genes encoding the key fatty acid desaturase enzymes determining the fatty acid composition of CSO, namely stearoyl-ACP Δ9-desaturase and oleoyl-PC Δ12-desaturase (also known as ω6-desaturase). Several candidate genes for these enzymes were first cloned from a cottonseed cDNA library based on their expected homology to the already sequenced Δ9-desaturase gene from castor bean and the Δ12-desaturase gene from *Arabidopsis thaliana*. Analysis of expression patterns for the candidate sequences revealed the particular genes that were responsible for the activity of these enzymes in the developing seed, namely the Δ9-desaturase gene *ghSAD-1* [35] and the Δ12-desaturase gene *ghFAD2-1* [36]. Inverted-repeat constructs encoding hpRNA targeted against either *ghSAD-1* or *ghFAD2-1* and driven by the seed-specific soybean lectin promoter were transformed into Coker 315 cotton. Silencing of *ghFAD2-1* expression resulted in greatly increased levels of oleic acid in more than half of the 29 individual transgenic lines examined, ranging up to 78% oleic acid in the mature seeds of primary transgenic plants compared to about 13% in seeds of untransformed plants (Table 2). Similarly,

Table 2. Fatty Acid Composition of Seed Oil from Coker 315 Cotton and Variants Derived from it by PTGS of Δ9- and Δ12-Desaturases

Genotype	Fatty acid composition (%)*			
	Palmitic	Stearic	Oleic	Linoleic
Normal (Coker 315)	26	2	13	59
High oleic (HO)	15	2	78	4
High stearic (HS)	15	40	4	39
HO & HS	14	40	37	6

* Minor fatty acids not reported.

silencing of the *ghSAD-1* gene resulted in increased levels of stearic acid in over half of 26 individual transgenic lines, the highest level being 40%, approximately twentyfold greater than the 2% present in untransformed control plants (Table 2). Similar changes in fatty acid composition were found in experiments using conventional antisense constructs targeted against the same genes, but at much lower frequencies than with the hpRNA-encoding constructs. Interestingly, the content of palmitic acid in both high-stearic (HS) and high-oleic (HO) lines was significantly and favorably reduced from 26% down to 15% (Table 2). As expected, these changes in fatty acid composition have proven to be heritable with the same extreme profiles being present in the progeny of the transgenic lines.

We have examined the possibility of producing further novel fatty acid compositions by intercrossing the most extreme initial HO and HS lines. A wide range of combinations of stearic, oleic and linoleic acids were evident in the F_2 generation of the cross. For example, one F_2 plant showing silencing of both $\Delta9$- and $\Delta12$-desaturases had 40% stearic acid and 37% oleic acid and retained the reduced level of palmitic acid (Table 2). Based on the pattern of variation in this cross, it now appears possible to develop a wide range of improved fatty acid profiles in CSO having greatly reduced contents of linoleic acid and palmitic acid and with various combinations of oleic and stearic acid as required for particular end uses. Furthermore, it is possible to extend this variation in fatty acid composition by choosing parental lines that have appropriate intermediate degrees of silencing of $\Delta9$- and $\Delta12$-desaturase genes.

COMMERCIAL EVALUATION AND VARIETY DEVELOPMENT

Samples of oils from the initial HO and HS cotton lines are currently undergoing detailed evaluation by food technologists to determine their physical properties, flavor characteristics and oxidative stability. Due to the replacement of most of the unstable linoleic acid with either oleic or stearic acid, these oils should have both excellent inherent stability and the required physical properties for various food industry applications, without the need for hydrogenation. Although it may be possible to further reduce linoleic acid below the 4% present in the best high-oleic lines, such a change may not be advantageous, since there is evidence from studies with mid-oleic genotypes of rapeseed [37] that a modest level of linoleic acid in the oil is desirable from a flavor standpoint and should not be sacrificed for the minimal further improvement in stability that would result from its complete removal. Additionally, by varying the relative proportions of oleic and stearic acid we anticipate that it should be possible to develop CSO with different physical properties, ranging from liquid oils to semi-solid fats. One future possibility is the development of structured triglycerides for use as specialty confectionery fats, for example as cocoa butter substitutes.

The eventual development of commercial cotton varieties producing nutritionally improved CSO will involve the incorporation of some additional features that should enhance their commercial potential. Firstly, further reductions in palmitic acid content are nutritionally desirable and are likely to be achievable without compromising stability in the high-oleic oil. Palmitic acid should be able to be reduced by either silencing of palmitoyl-ACP thioesterase gene or genetic enhancement of β-keto-acyl synthase (KASII) activity [38]. Secondly, because the new oils will be regarded as genetically-modified (GM) foods, it may be beneficial to minimize the amount of introduced DNA. The high frequency of silencing reliably obtained using hpRNA makes it feasible to introduce the silencing construct and the selectable marker gene independently [39], thus allowing the selectable marker gene to be subsequently removed by genetic segregation. Furthermore, additional enhancements in hpRNA-mediated gene silencing techniques achieved since the creation of the initial lines will enable the same fatty acid modifications to be achieved using constructs that contain only very small fragments of DNA (approximately 100 nucleotides) from the untranslated region of the target desaturase gene and driven by a cottonseed promoter [40]. Thus it appears possible to develop readily high-stearic and high-oleic cottonseed that will contain only minimal amounts of re-introduced cotton DNA and no selectable marker gene. Such an approach should maximize the chances of commercial success for the product in the current environment of uncertainty about consumer acceptance of GM food products.

CONCLUSION

Gene technology has provided plant breeders with powerful new tools for manipulating the composition of plant products. In oilseeds, this has significantly extended the capability to achieve major alterations in the relative proportions of the fatty acids present in the oil, for the purposes of improving nutritional value without compromising functionality. Oilseeds that are rich in stearic or oleic acid will provide commercial cooking fats and oils with the required stability and performance and enable industry to move away from the use of oils with high contents of palmitic acid and *trans*-fatty acids. The wide-scale introduction of these improved oils has the potential to deliver substantial public health benefits through lowering of serum LDL-cholesterol levels and consequent reduced incidence of cardiovascular disease. Such nutritionally-enhanced oils will be some of the first food products to be genetically modified specifically to provide consumer benefits and are considered likely to have greater consumer appeal than foods derived from initial GM crops that were engineered for predominantly agricultural benefits.

ACKNOWLEDGMENT

This cottonseed oil research outlined here was sponsored by the Australian Cotton R&D Corporation (Grant No. CSP-78C).

REFERENCES

1. Krawczyk T: Fat in dietary guidelines around the world. INFORM 12:132–138, 2001.
2. Stamler J, Shekelle R: Dietary cholesterol and human coronary heart disease. Arch Pathol Lab Med 112:1032–1040, 1988.
3. Sleight P: Cholesterol and coronary heart disease mortality. Austr New Zealand J Med 22 (Suppl):576–579, 1992.
4. Smith GD, Song F, Sheldon TA: Cholesterol lowering and mortality. Brit Med J 306:1367–1367, 1993.
5. Mensink RP, Katan MB: Effect of dietary fatty acids on serum lipids and lipoproteins. A meta-analysis of 27 trials. Arterioscler Thromb 12:911–919, 1992.
6. Zock PL, de Vries JHM, Katan MB: Impact of myristic versus palmitic acid on serum lipid and lipoprotein levels in healthy women and men. Arterioscler Thromb 14:567–575, 1994.
7. Bonanome A, Grundy SM: Effect of dietary stearic acid on plasma cholesterol and lipoprotein levels. N Eng J Med 318:1244–1248, 1988.
8. Dougherty RM, Allman MA, Iacono JM: Effects of diets containing high or low amounts of stearic acid on plasma lipoprotein fractions and fecal fatty acid excretion of men. Am J Clin Nutr 61:1120–1128, 1995.
9. Chang SS, Peterson RJ, Ho CT: Chemical reactions involved in the deep-fat frying of foods. J Am Oil Chem Soc 55:718–727, 1978.
10. Warner K, Neff WE, Byrdwell WC, Gardner HW: Effect of oleic and linoleic acids on the production of deep-fried odor in heated triolein and triolinolein. J Agri Food Chem 49:899–905, 2001.
11. Williams MJ, Sutherland WH, McCormick MP, de Jong SA, Walker RJ, Wilkins GT: Impaired endothelial function following a meal rich in used cooking fat. J Am Coll Cardiol 33:1050–1055, 1999.
12. Billek G: Health aspects of thermoxidized oils and fats. Euro J Lipid Sci Techn 102:587–593, 2000.
13. Ray JD, Carr BT: Empirical modeling of soybean oil hydrogenation. J Am Oil Chem Soc 62:1218–1222, 1985.
14. Ray JD: Behavior of hydrogenation catalysts. I. Hydrogenation of soybean oil with palladium. J Am Oil Chem Soc 62:1213–1217, 1985.
15. Mensink RP, Katan MB: Effect of dietary *trans* fatty acids on high-density and low-density lipoprotein cholesterol levels in healthy subjects. N Engl J Med 323:439–445, 1990.
16. Noakes M, Clifton PM: Oil blends containing partially hydrogenated or interesterified fats: differential effects on plasma lipids. Am J Clin Nutri 98:242–247, 1998.
17. Zock PL, Katan MB: Hydrogenation alternatives: effects of *trans* fatty acids and stearic acid versus linoleic acid on serum lipids and lipoproteins in humans. J Lipids Res 33:399–410, 1992.
18. Ascherio A, Willett WC: Health effects of *trans* fatty acids. Am J Clin Nutri 66(4S):1006S–1010S, 1997.
19. Hulshof KFAM, van Erp-Baart MA, Anttolainen M, Becker W, Church SM, Couet C, Hermann-Kunz E, Kesteloot H, Leth T, Martins I, Moreiras O, Moschandreas J, Pizzoferrato L, Rimestad AH, Thorgeirsdottir H, van Amelsvoort JMM, Aro A, Kafatos AG, Lanzmann-Petithory D, van Poppel G: Intake of fatty acids in Western Europe with emphasis on *trans* fatty acids: The TRANSFAIR study. Eur J Clin Nutr 53:143–157, 1999.
20. UK Ministry of Agriculture, Fisheries and Food: Household Food Consumption and Expenditure 1994. London: HMSO, 1995.
21. Knowles PF, Hill AB: Inheritance of fatty acid content in the seed oil of a safflower introduction from Iran. Crop Sci 4:406–409, 1964.
22. Moore KM, Knauf DA: The inheritance of high oleic acid in peanut. J Hered 80:252–253, 1989.
23. Soldatov KI: Chemical mutagenesis in sunflower breeding. In "Proceedings of the 7th International Sunflower Conference, Krasnodar, USSR 27 June–3 July 1976." Vlaardingen, The Netherlands: International Sunflower Association, pp 352–357, 1976.
24. Fitch-Haumann B: Modified oil may be key to sunflower's future. INFORM 5:1198–1210, 1994.
25. Fehr WR, Welke GA, Hammond EG, Duvick DN, Cianzio SR: Inheritance of reduced palmitic acid content in seed oil of soybean. Crop Sci 31:88–89, 1991.
26. Graef GL, Fehr WR, Hammond EG: Inheritance of three stearic acid mutants of soybean. Crop Sci 25:1076–1079, 1985.
27. Osorio J, Fernandez-Martinez J, Mancha M, Garces R: Mutant sunflowers with high concentration of saturated fatty acids in the oil. Crop Sci 35:739–742, 1995.
28. Waterhouse PM, Wang MB, Lough T: Gene silencing as an adaptive defence against viruses. Nature 411:834–842, 2001.
29. Knutzon DS, Thompson GA, Radke SE, Johnson WB, Knauf VC, Kridl JC: Modification of *Brassica* seed oil by antisense expression of a stearoyl-acyl carrier protein desaturase gene. Proc Nat Acad Sci USA 89:2624–2628, 1992.
30. Kinney AJ: Development of genetically engineered soybean oils for food applications. J Food Lipids 3:273–292, 1996.
31. Stoutjesdijk PA, Hurlstone C, Singh SP, Green AG: High-oleic Australian *Brassica napus* and *B. juncea* varieties produced by co-suppression of endogenous Δ12-desaturases. Biochem Soc Trans 28:938–940, 2000.
32. Hamilton AJ, Brown S, Yuanhai H, Ishizuka M, Lowe A, Solis AGA, Grierson D: A transgene with repeated DNA causes high frequency, post-transcriptional suppression of ACC-oxidase gene expression in tomato. Plant J 15:737–746, 1998.
33. Smith NA, Singh SP, Wang MB, Stoutjesdijk PA, Green AG, Waterhouse PM: Total silencing by intron-spliced hairpin RNAs. Nature 407:319–320, 2000.
34. Liu Q, Singh S, Green A: Genetic modification of cotton seed oil using inverted-repeat gene-silencing constructs. Biochem Soc Trans 28:927–929, 2000.
35. Liu Q, Singh SP, Sharp PJ, Green AG, Marshall DR: Nucleotide sequence of a cDNA from *Gossypium hirsutum* encoding a stearoyl-acyl carrier protein desaturase (Accession No. X95988) (OGR 96-012). Plant Physiol 110:1435, 1996.
36. Liu Q, Singh SP, Brubaker CL, Sharp PJ, Green AG, Marshall DR: Molecular cloning and expression of a cDNA encoding a microsomal ω-6 fatty acid desaturase in cotton (*Gossypium hirsutum* L.). Aust J Plant Physiol 26:101–106, 1999.
37. Xu XQ, Tran VH, Palmer MV, White K, Salisbury P: Chemical,

physical and sensory properties of Monola oil, palm olein and their blends in deep frying trials. Food Australia 52:77–82, 2000.
38. Broun P, Gettner S, Somerville C: Genetic engineering of plant lipids. Ann Rev Nutr 19:197–216, 1999.
39. Lu H-J, Zhou X-R, Gong Z-X, Upadhyaya N: Generation of selectable marker-free transgenic rice using double right-border (DRB) binary vectors. Aust J Plant Physiol 28:241–248, 2001.
40. Wesley SV, Helliwell CA, Smith NA, Wang M-B, Rouse DT, Liu Q, Gooding PS, Singh SP, Abbott D, Stoutjesdijk PA, Robinson SP, Gleave AP, Green AG, Waterhouse PM: Construct design for efficient, effective and high-throughput gene silencing in plants. Plant J 27:581–590, 2001.

Received February 5, 2002.