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Biotechnology of Oilseeds

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Introduction

Oilseeds are among the most ancient crops domesticated by mankind. One of the first oilseed crops to be cultivated systematically was probably linseed. There is fossil evidence of the selection for agriculture of oil-bearing varieties of linseed from over 8000 years ago. Their first mention in written records is found in ancient Sumerian and Akkadian texts dating from 4000 to 5000 years ago, which refer to oilseeds such as sesame and linseed (Helbaek, 1966). From the beginning of their cultivation, oilseeds were utilized in a variety of edible and non-edible applications. For example, the ancient Persians used sesame oil in cooking, as a body massage, for illumination, in cosmetics and as a lubricant in simple machines.

The relative proportion of oilseed products utilized for non-edible applications began to decline in the early twentieth century, due to the ready availability of large amounts of inexpensive mineral oil derived from fossilized material. More recently, however, the large increases in the price of fossil-derived mineral oils and the recognition that this is a limited resource have focused attention once more on the potential non-food uses of vegetable oils. The market for edible vegetable oils has also increased substantially since the Second World War. This is due to a number of factors, including improvements in agriculture, rising levels of affluence in many consuming countries and the trend away from animal-derived fats for medical or dietary reasons.

The net effect of these various trends has been an enormous increase in the demand for oilseeds and their products, particularly in Europe and the Americas. In the past decade alone, world oilseed production has increased

Abbreviations: ABA, abscisic acid; ACP, acyl carrier protein; CDP, cytidinediphosphate; DAG, diacylglycerol; DAG-AT, diacylglycerol acyltransferase; EMS, ethyl methane sulphonate; ER, endoplasmic reticulum; G3P, glycerol-3-phosphate; G3P-AT, glycerol-3-phosphate acyltransferase; KAS I, 3-ketoacyl-ACP synthase I; KAS II, 3-ketoacyl-ACP synthase II; LEA, late-embryo-abundant protein; LPA, *lyso*-phosphatidic acid; LPA-AT, *lyso*-phosphatidic acid acyltransferase; LPC, *lyso*-phosphatidylcholine; LPC-AT, *lyso*-phosphatidylcholine acyltransferase; PC, phosphatidylcholine; TAG, triacylglycerol.

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by more than one-third, from about 160 MT to 215 MT. As much as one-third of the vegetable oil produced is used for non-edible, industrial purposes (Battey, Schmid and Ohlrogge, 1989). The increasing demand for high-quality oilseeds, designed for specific market applications, both edible and non-edible, has coincided with the emergence of new technologies for crop modification. Efforts are now underway in many different countries, aimed at the production of specialized 'designer' oilseed crops. The oil compositions of such crops will be manipulated in order that different crop species or varieties can each be targeted towards a particular commercial sector, for example high oleic edible oils or high linoleic edible oils for edible use, long-chain oils for use as lubricating fluids, short-chain oils for use as detergents, mono-unsaturated oils for use in polymer synthesis, hydroxylated oils for use in lubricants, paints and polymers, and so on.

Four crops—soybean, sunflower, rape and palm—provide over 70% of the world's vegetable oil supplies (Scowcroft, 1990) with cottonseed, coconut and groundnut providing most of the remainder. There are many other sources of vegetable oils, such as olives, grape seed or tomato seed, but these are usually used near the locality where the crops are grown. A detailed and comprehensive account of the cultivation, breeding and uses of all these oilseeds is given in a recent book (Downey, Robbelen and Ashri, 1989). Most vegetable oil is produced for human consumption but about 20–30% is used for non-edible purposes. Oil is not the only economically valuable product of oilseed crops, and most of the other components are sold after processing. The protein meal from the seeds is usually recovered and sold as animal feedstuff. The hulls of the seeds are also used variously in animal feed, as fuel, or in the construction of building or insulation boards. Lecithin is another small but valuable component. In this review the term 'oilseed crops' refers to all crops from which oil is extracted for further use. For some crops, however, oil is not the main product. In soybean, protein is at least as important. For corn (*Zea mays*), starch is the major product for which the crop is grown, mainly for conversion to high-fructose corn syrup. If the oil content of corn rises above just a few per cent, the conversion of the starch is interfered with, so corn varieties with higher oil contents are not grown. Cotton is another example where oil is secondary to the main product of fibre for which cotton is mainly grown.

Some aspects of the genetic engineering of oil quality in oilseeds have been dealt with in the past few years (Knauf, 1987; Battey, Schmid and Ohlrogge, 1989; Somerville and Browse, 1991). In this review, we will consider the application of biotechnology not only to the oil component of oil seeds but also to the protein and other products of potential interest in the future. Lipase is one such product which can be extracted from the seeds of castor bean or from the seedlings of many oilseed plants. This has possible applications in catalysing reactions of lipid substrates dissolved in organic solvents. Oleosins, which surround the oil droplets in the seed and prevent coalescence of the oil in the dry seed, might have uses as emulsifiers.

Uses of vegetable oils

Most vegetable oil is used directly in the human diet as cooking or salad oil and in margarine, or indirectly in processed products such as shortenings and confectionary. There is also a multiplicity of uses of vegetable oils for non-edible purposes, although the amounts involved are often quite small. The major non-edible end use of vegetable oils is in the production of soaps and detergents from palm and coconut oils. Other non-edible uses of vegetable oils are in lubricants, plastics and resins, paints, varnishes and coatings, cosmetics and as precursors to a wide range of chemicals. Three examples of such oils are those derived from linseed, castor and jojoba seeds. Linseed oil, which contains large amounts of α-linolenic acid, is used in coatings and drying agents because the double bonds of α-linolenic acid are very susceptible to oxidation, leading to polymerization, and yielding a solid but soft and flexible product. Castor oil, which contains hydroxylated fatty acids, is used in paints and coatings but also has uses in lubricants, and as a precursor to plastics manufacture. Jojoba oil, which is a liquid wax rather than a triacylglycerol oil, is used in lubricants and cosmetics. It has been reported that it is possible to obtain wax esters with very similar properties to jojoba wax from mustard oil by esterification using a lipase in organic solvents (Mukherjee and Kiewitt, 1988). Such oils can be used directly, or they may be cleaved to partial glycerides for use as emulsifying agents in margarines, or completely cleaved to fatty acids. During the past 10 years, the use of lipases to catalyse such reactions, particularly with higher value oils, has become a reality (Mukherjee, 1990). Using lipases may significantly reduce production costs compared to conventional chemical engineering methods. The oils or fatty acids are often chemically processed by one or more of a wide range of reactions such as hydrogenation, amidation, amination, ozonolysis or epoxidation, depending on the end use. The non-food uses of vegetable oils and the chemical conversions are described in depth elsewhere (Pryde and Rothfus, 1989). It has been estimated that the volume of vegetable oil used for the production of chemicals would double if full use was made of them, rather than using petrochemicals as the raw material (Pryde and Rothfus, 1989).

Changes in the quality of oilseeds sought by users

Changes in oil quality, i.e. the type of fatty acids contained in the triacylglycerols, are desired for both edible and non-edible oils, though it is in the oils for industrial purposes that the most radical alterations are wanted and it is the accomplishment of these changes that will require genetic engineering.

EDIBLE OILS

Polyenoic fatty acids

The double bonds of polyenoic fatty acids such as α-linoleic and linolenic are susceptible to oxidation by molecular oxygen, leading to the formation of

Table 1. Fatty acid nomenclature. Trivial names for fatty acids have been used throughout the manuscript but sometimes the abbreviated designations given below appear. The shorthand designation refers to the number of carbons in the hydrocarbon chain:number of double bonds (see Gunstone, 1986 for more details)

Trivial name	Shorthand designation
lauric	12:0
palmitic	16:0
stearic	18:0
oleic	18:1
linoleic	18:2
linolenic	18:3
gadoleic	20:1
erucic	22:1
nervonic	24:1
ricinoleic	18:1-OH

strongly flavoured compounds which are undesirable in edible oils. The flavour of oxidized α-linolenic acid is particularly strong. Soybean and rape oils contain significant amounts of α-linolenic acid, so decreases in this fatty acid are sought in order to increase the oxidative stability of the oil and thereby improve flavour. The seeds of legumes, such as soybean, also contain lipoxygenases which cause the oxidation of α-linolenate moieties, so breeding and genetic engineering strategies are being devised to eliminate these enzymes. The requirement for hydrogenation could also be reduced, hence minimizing the levels of undesirable *trans* unsaturated fatty acids often present in hydrogenated edible oils. The possible health risks of high levels of dietary *trans* unsaturated fatty acids in some vegetable oil products is currently attracting adverse publicity and any reduction in their amounts would therefore be welcomed by the food industry.

Palmitic acid

A decrease in the levels of palmitic acid in soybean oil is also wanted for health reasons. Excessive dietary intake of this acid has been implicated as a contributory factor in the aetiology of coronary heart disease. At the same time, increases in the levels of stearic acid (which is not believed to have the same attached health risks) are sought. Small increases in the levels of palmitic acid in rape oil are wanted to allow the production of 100% rape oil margarine. High levels of C18 fatty acids in present varieties of rape oil cause crystallization of the oil in the margarine, leading to an unacceptably grainy texture which can only be eliminated by the addition of other types of vegetable oils, e.g. sunflower or soybean, which contain higher levels of palmitate.

Cocoa butter

Cocoa butter substitutes have been sought for use in confectionary since the genuine material is relatively expensive. Substitutes can be made by interesterification of cheap palm oil with stearic acid by using an *sn* -1,3 specific lipase from *Rhizopus* as catalyst (Macrae and Hammond, 1985). A problem here is

that such substitutes, although they are derived from natural oils and are chemically very similar to cocoa butter triacylglycerols, may only be used as a minor admixture in chocolate products. Hence the use of cocoa butter substitutes is currently limited by labelling regulations, particularly in Europe and N. America.

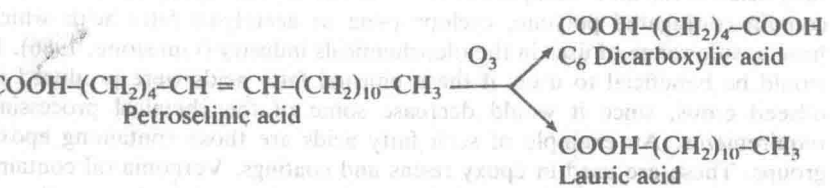
NON-EDIBLE OILS

Lauric acid

Medium-chain-length fatty acids (mainly lauric acid) in seeds of a crop which can be grown in temperate regions are wanted by the detergent industry in Europe and N. America in order to replace or augment the present source of such fatty acids, i.e. imported coconut or palm kernel oils. *Cuphea* oils are also potentially useful in this regard since they contain higher levels of the medium- and short-chain fatty acids than do the tropical palm oils. The production of detergents is the single most important end use of a vegetable oil for non-edible purposes; thus the production of lauric oils within the EEC or US and the development of a domestic source of such oils would save considerable sums of money on the trade balance of these countries. Conversely, of course, this could have a negative effect on those, mainly developing, countries which rely on exporting lauric oils to accrue foreign currency.

Petroselinic acid

An alternative for obtaining lauric acid to that described above is the possibility of growing oilseed crops containing petroselinic acid in the seed oil, since the double bond in the Δ^6 position can be cleaved by ozonolysis to yield C12 and C6 units, as shown below.



This reaction yields lauric acid for detergent applications, and also a C6 dicarboxylic acid, which is eminently suitable for use as a monomer in the manufacture of a wide range of industrial polymers, particularly nylons.

Hydroxylated fatty acids

Hydroxylated fatty acids, such as ricinoleic acid, are sought in the seed oils of temperate species since the main source of this acid is castor oil which is

imported from tropical countries where supplies are often uncertain for climatic or other reasons. Ricinoleic acid from castor oil is particularly useful because the OH group in the Δ^{12} position and the double bond in the Δ^{9} position allow many chemical conversions to be carried out which are not easily achieved with oils from other plants. Ricinoleic acid is used in lubricants, plasticizers, coatings, surfactants and pharmaceuticals. Derivatives are also used in polyester and other polymer manufacture, in cosmetics, non-drip paints and in greases.

Homogeneous triacylglycerols

Most seed oils contain triacylglycerols with a highly heterogeneous fatty acid composition. Since the end use of many seed oils depends upon only one of these fatty acids, the production of triacylglycerols containing as much of one fatty acid as possible is a major goal of the oilseed biotechnologist. This would make the extraction of relatively pure fatty acids from the oil much easier and more commerically attractive. An already achieved example of this is the breeding of sunflower cultivars that contain greater than 90% oleic acid. A trierucoylglycerol would make processing of erucic acid from high erucic acid rape oil or other sources much more commercially attractive. The main seed oils containing erucic acid, such as rape or mustard, contain this fatty acid almost exclusively in the *sn*–1 and *sn*–3 positions so that a theoretical maximum of less than 70% is achievable at present. Oils containing erucic acid may be employed directly as lubricants, or the erucic acid can be converted by ozonolysis and amidation to monomers, for use in plastic or nylon manufacture.

Substituted fatty acids

The production of seed oils containing any of a wide variety of substituted fatty acids would give savings to industry on the costs of processing simple fatty acids to the more complex ones. Plants have been found whose seed oils contain conjugated polyene, cyclopropene or acetelynic fatty acids which have a wide range of uses in the oleochemicals industry (Gunstone, 1986). It would be beneficial to users if these unusual fatty acids were produced in oilseed crops, since it would decrease some of the chemical processing requirements. An example of such fatty acids are those containing epoxy groups. These are used in epoxy resins and coatings. Vernonia oil contains such fatty acids but since Vernonia has not yet been developed as an oil seed crop, the epoxy fatty acids are currently obtained by epoxidation of soybean oil.

Oil synthesis

In order to set the biotechnological manipulation of oil quality in context, the metabolic pathways involved in oil synthesis will be described briefly. For more detailed description of lipid and storage oil metabolism, the reviews by

Roughan and Slack (1982) and Harwood (1988) give a comprehensive account. Here we shall emphasize the recent research targeted at the molecular genetics of storage lipid synthesis and aspects of the metabolic control of oil production. Most seed storage oils are composed of triacylglycerols, with the notable exception of jojoba, which stores wax esters. The synthesis of triacylglycerols occurs in three main stages; first the synthesis of fatty acids (palmitic, stearic and oleic) in the plastid (*Figure 1*). Oleic acid can then, depending on the plant species, be metabolized to other fatty acids by desaturation, elongation, hydroxylation or other reactions, such as epoxidation, to create modified fatty acids. In the final stage of oil synthesis, the various fatty acids form an acyl-CoA pool, which is thought to be in the endoplasmic reticulum (ER) where they are drawn upon by acyltransferases of the Kennedy pathway to form triacylglycerols (*Figure 2*).

FATTY ACID SYNTHESIS

Fatty acids in plants are synthesized mainly in the plastid (Harwood, 1988). The first committed step of fatty acid biosynthesis is that catalysed by acetyl-CoA carboxylase to create malonyl-CoA (Slabas and Hellyer, 1985). There is some correlative evidence from castor that acetyl-CoA carboxylase is rate-limiting in the synthesis of fatty acids (Simcox *et al.*, 1979). In rape, the measurable activities are low but increase dramatically as oil synthesis begins (Turnham and Northcote, 1983). The notion that acetyl-CoA carboxylase is the major regulatory step of fatty acid synthesis has been strengthened by recent experiments where levels of the intermediates of fatty acid synthesis were measured under conditions of low and high pathway flux (Post-Beittenmiller, Jaworski and Ohlrogge, 1991). These experiments are described in detail on p. 12. Fatty acids are then synthesized by the sequential addition of two carbon units from malonyl-CoA to the acyl chain (*Figure 1*) by a type II fatty acid synthetase. Fatty acid synthetase II is a system consisting of six enzymatic activities, which, although each can be isolated as individual enzymes, is thought to act as a single complex *in vivo*. The lengthening acyl groups are attached to acyl carrier proteins (ACPs) throughout *de novo* fatty acid synthesis.

Acyl carrier proteins

Acyl carrier proteins and their genes are the most thoroughly characterized of the components of the fatty acid and oil synthesis pathways. ACPs are acidic, low molecular weight proteins and are essential co-factors for at least 12 enzymes involved in fatty acid metabolism. Much research effort has been focused on ACP since it is likely that information about the control of expression of ACP in seeds will shed light on the regulation of genes involved with oil synthesis in general during embryo development (Ohlrogge *et al.*, 1987). Immunogold localization studies have shown that ACP is almost exclusively localized to the plastids of rape leaves, where the majority of the ACP was found to be associated with the thylakoid membranes (Slabas and