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Biotechnological Strategies for the Modification of Food Lipids

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Introduction

Now that the nutritional requirements of infants, adults and patients are becoming more clearly defined, biotechnology is moving to the forefront of lipid modification strategies. Medium chain fatty acids are an important source of rapid energy for pre-term infants and for patients with fat malabsorption-related diseases. Polyunsaturated fatty acids (PUFAs) are important both for infant development and disease prevention in adults. Lipase-catalysed interesterification has been used to produce structured lipids containing medium chain fatty acids and PUFAs. These techniques have also been used to increase PUFA concentrations in fish oils, and to introduce these fatty acids into vegetable oils. Genetic engineering of oilseed plants can be used to develop plants which produce medium chain fatty acids and PUFAs. By manipulating growth conditions, high-PUFA oils can also be obtained from algae and fungi. Even though biotechnological processes applied to the modification of the structure and properties of fats and oils are expensive relative to chemical processes, their use may be justified due to their greater lipid tailoring potential.

Biotechnology, as it applies to fats and oils, involves the production of nutritionally improved products through enzymatic modifications, biotransformations and genetic engineering. Recent trends in health-related concerns about fats and oils have included decreasing consumption of fats high in saturated and *trans* unsaturated fatty acids as well as increasing consumption of essential and polyunsaturated fatty acids. Lipase-catalysed reactions, biotransformations and genetic engineering of plants are all strategies being used to develop fats and oils with increased concentrations of medium chain, essential and polyunsaturated fatty acids. The products manufactured by these processes have been used to meet infant fat nutritional requirements and for disease treatment and prevention in adults. These technologies have also been used to modify the physical properties of certain lipids, including their melting profiles and textural properties.

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Medium chain fatty acids (MCFAs) have been used in the treatment of fat malabsorption related diseases and as a significant source of energy for preterm infants. Consumption of fats and oils containing polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), is increasing due to their role in preventing atherosclerosis in adults. The concentration of these fatty acids as well as their positional distribution within a triacylglycerol molecule are important factors.

Typically, chemical methods of lipid modification have been used in industrial applications due to their lower cost. However, biotechnology allows production of fats and oils with specific compositions, both in terms of positional distribution of specific fatty acids and fatty acid composition.

The aim of this paper is to provide an overview of how biotechnology, in the form of lipase-catalysed reactions, biotransformations and genetic engineering of oilseeds allow both nutritional and physical modification of fats and oils.

Metabolism and absorption of fats and oils

The positional distribution and fatty acid composition of fats and oils can have an impact on their digestion and absorption (*Figure 1*). Differences in the requirements for specific structures and composition of fats and oils are due to differences between adult and infant digestive systems. The less mature infant digestive system possesses reduced levels of pancreatic lipase and bile salts, and greater lingual lipase activity (Zoppi *et al.*, 1972; Fredrikzon and Olivecrona, 1978; Watkins, 1988).

Lingual lipase, which accounts for 50 to 70% of lipid hydrolysis in infants (Watkins, 1988), but has minimal importance in adults' digestion, is active in the upper intestinal tract, hydrolysing triacylglycerols (TAGs) to monoacylglycerols (MAGs), diacylglycerols (DAGs), and free fatty acids (FFAs). It is more specific towards short and medium chain fatty acids and fatty acids in the sn-3 position, producing mainly 1,2-DAGs and FFAs (Christensen *et al.*, 1995; Small, 1991). Gastric lipase in the stomach is also more specific towards short and medium chain fatty acids and continues to hydrolyse positions 1 and 3 of the TAGs to produce FFAs, MAGs and DAGs. Short and medium chain fatty acids, including butyric, caproic, caprylic and capric acid are more soluble in aqueous media and can be absorbed through the stomach directly into venous circulation, travelling via the portal vein to the liver where they are oxidized and used as a rapid source of energy (Borum, 1992; Nelson, 1992). This attribute is particularly important for treatment of fat malabsorption related diseases and in the provision of a rapid source of energy for premature infants. Medium chain fatty acids also provide a rapid source of energy in muscle since their transport into the mitochondria for β-oxidation is not carnitine dependent (Bruckner, 1992).

Pancreatic lipase is present in the small intestine and provides the final hydrolysis of TAGs. It is 1,3-specific, with a slight preference towards fatty acids in the sn-1 position (Small, 1991; Berdanier, 1995). It is more active towards short and medium chain fatty acids and has low activity with respect to long chain PUFAs in the sn-1 and sn-3 positions (Christensen *et al.*, 1995).

After hydrolysis, the fatty acids and 2-MAGs in the form of micelles with bile salts are absorbed through the intestinal mucosa. The positional distribution of fatty acids

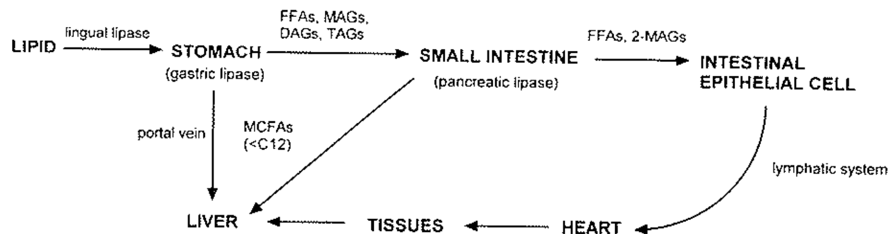

Figure 1. Metabolism and transport of lipids and their hydrolysis components in humans.

can have a dramatic impact on their degree of absorption. Long chain saturated fatty acids such as palmitic acid are poorly absorbed in their free acid form since they are solid at body temperature and form insoluble calcium and magnesium soaps in the intestine. As a 2-MAG, palmitic acid is easily absorbed in the intestine (Innis *et al.*, 1995; Tomarelli, 1988), which is important for infants since human milkfat contains 20 to 30% palmitic acid, 70% of which is present in the sn-2 position (Innis *et al.*, 1995). Placement at the sn-2 position is also important for long chain PUFAs, which are poor substrates for pancreatic lipase and are better absorbed as 2-MAGs, requiring hydrolysis by carboxyl ester hydrolases when present in positions sn-1 and 3 (Small, 1991).

New TAGs, which are formed by reacylation of FFAs and 2-MAGs after absorption from the intestines, are combined with phospholipids and apolipoproteins to form chylomicrons for transport through the lymphatic system and into general circulation. Any short and medium chain fatty acids which were not absorbed through passive diffusion into venous circulation as well as monoacyglycerols containing these fatty acids, are also incorporated into chylomicrons and are transported through the lymphatic system (Lambert *et al.*, 1996; Bell *et al.*, 1997).

Upon transport to adipose and muscle tissue, lipoprotein lipase at the surface of capillary endothelial cells hydrolyses TAGs to 2-MAGs and FFAs, which are then transported into cells, undergoing reacylation to form TAGs for storage (Small, 1991; Tso and Wediman, 1987).

Overall, proper and efficient absorption is dependent on the composition and positional distribution of fatty acids. Therefore, any attempts to provide nutritionally improved fats and oils for applications in infant formulas, disease treatment and adult disease prevention must account for differences in metabolism as well as differences in the nutritional effects of specific fatty acids on different populations of consumers.

Metabolic significance of specific fat components

The major fatty acids associated with biotechnological modifications of fats and oils are MCFAs and long chain PUFAs. Genetic engineering of oilseed crops as well as lipase-catalysed production of structured lipids are the predominant methods of increasing the MCFA concentration. Long chain PUFAs are concentrated in fish oils and incorporated into vegetable oils by genetic engineering of oilseed crops and lipase-catalysed interesterification reactions.

MEDIUM AND LONG CHAIN SATURATED FATTY ACIDS

As previously discussed, MCFAs are important to infants and adults, for varied reasons. Infants use 25% of their caloric intake for growth (Hardy and Kleinman, 1994), and for those who are premature or have reduced biliary flow, MCFAs are an important source of energy (Borum, 1992). Medium chain fatty acids possess several advantages over other fatty acids which has increased the interest in developing new, or modifying existing fats and oils to increase their concentrations (*Table 1*). Unmodified sources of MCFAs include coconut and palm kernel oils, which lack significant concentrations of essential and polyunsaturated fatty acids, making them unsuitable for general use. Modified lipids containing increased concentrations of MCFAs have been used in the treatment of diseases where fat malabsorption and metabolism are a problem, such as AIDS, cystic fibrosis, cirrhosis and anorexia (Fan, 1997; Sandström *et al.*, 1993; Bell *et al.*, 1997; Ulrich *et al.*, 1996). Medium chain fatty acids are also essential components of formulas for premature infants (40–50% of total fatty acids) and parenteral and enteral formulations in Europe, where coconut oil is used as a source of medium chain TAGs (Mascioli *et al.*, 1988; Ulrich *et al.*, 1996; Borum, 1992). In terms of improved nutrition, MCFAs have been used as an energy supplement for athletes (Van Zyl *et al.*, 1996).

The disadvantages associated with medium chain TAGs include the potential for acidosis or toxicity at high concentrations, their negative effect on plasma concentrations of other fatty acids and their lack of essential and polyunsaturated fatty acids (Carnielli *et al.*, 1996; Sandström *et al.*, 1993; Bell *et al.*, 1997; Ulrich *et al.*, 1996).

The use of biotechnology to modify existing fats and oils and incorporate MCFAs allows producers to avoid some of the disadvantages associated with medium chain TAGs. Lipase-catalysed interesterification reactions and genetic engineering of oilseeds allow production of TAGs containing a combination of medium chain, essential and polyunsaturated fatty acids. Both of these methods also allow some degree of control over the positional distribution of these fatty acids, making their use superior to simple blending of MCTs and long chain TAGs and chemical means of lipid modification (Sandström *et al.*, 1993; Nordenström *et al.*, 1995; Jeevanandam *et al.*, 1995).

The positive health effects of long chain saturated fatty acids are really only associated with infant development. Human milk contains 20 to 30% palmitic acid, 70% of which is present in the sn-2 position of the triacylglycerol (Innis *et al.*, 1995).

As a 2-MAG, palmitic acid is an important source of energy for infants, since it is only minimally absorbed in its free acid form, forming insoluble salts with calcium (Innis *et al.*, 1995; Small, 1991; Tomarelli, 1988). A higher degree of incorporation of palmitic acid into plasma TAGs has been observed for infants fed human milk compared to a vegetable oil based formula, where the majority of palmitic acid is in the sn-1 or sn-3 positions of the triacylglycerol (Innis *et al.*, 1994; Carnielli *et al.*,1995). Increasing the concentration of palmitic acid in the sn-2 position has also been associated with a small but significant increase in total energy absorption and reduced intestinal length required for absorption in rats (de Fouw *et al.*, 1994).

In terms of adult nutrition, it is less desirable to have long chain saturated fatty acids in the sn-2 position due to their possible hypercholesteremic effects and increased risk of heart disease (Caggiula and Mustad, 1997). Despite some concerns, not all saturated fatty acids have such a negative impact on health. While lauric, myristic and

Table 1. Metabolic and digestive advantages associated with the consumption of medium chain fatty acids

Advantages	Reference
Extremely resistant to oxidation and stable at high and low temperatures	Megremis (1991)
Most are not incorporated into chylomicrons, do not undergo desaturation or elongation and are therefore more likely to be used for energy	Babayan and Rosenau (1991)
As TAGs, are metabolized as quickly as glucose, yet have twice the energy density of carbohydrates	Bell *et al.* (1997)
Do not promote the synthesis of eicosanoids and are not involved in free radical formation, both of which are involved in inflammatory responses	Ulrich *et al.* (1996)
Are readily oxidized for rapid energy production	Bach *et al.* (1988); Johnson *et al.* (1990)
Are easily absorbed, are completely metabolized in the liver, do not interfere with the reticuloendothelial system and are not carnitine dependent for transport into mitochondria	Borum (1992); Sandström *et al.* (1993); Mascioli *et al.* (1988)

palmitic acid are considered to be hypercholesteremic (Denke and Grundy, 1992; Zock *et al.*, 1994), with palmitic acid being less hypercholesteremic than myristic acid, stearic acid seems to have the same or a better lowering effect on total and LDL cholesterol as oleic acid (Kris-Etherton and Yu, 1997; Pai and Yeh, 1997). Therefore, the positional distribution requirements for long chain saturated fatty acids is different for infants and adults, with a sn-2 positioning ideal for infants and positioning in the sn-1 and sn-3 positions preferable for adults.

LONG CHAIN UNSATURATED AND POLYUNSATURATED FATTY ACIDS

While the issue of *trans* fatty acids (TFAs) is important in terms of its negative health effects, it is not dealt with by biotechnologically-related lipid modifications, except in the production of low *trans* fatty acid margarines to replace those that have been chemically hydrogenated, and may contain 5 to 50% *trans* fatty acids (Ohlrogge, 1983). *Trans* fatty acids seem to be metabolized as efficiently as other fatty acids, but may impair desaturation and elongation of LA to arachidonic acid (AA), thereby affecting eicosanoid production and growth (Desci and Koletzko, 1995; Ratnayake and Chen, 1996). The negative effects of TFAs on adults are decreased concentrations of high density lipoprotein (HDL) cholesterol and increased concentrations of low density lipoprotein (LDL) cholesterol, producing a hypercholesteremic effect (Mensink and Katan, 1990; Zock and Katan, 1992). Despite concerns about the effects of TFAs in human milk and infant formulas on infant health, a causal link between TFAs and infant development has not been established, with only a possible association between TFAs and lower n-3 and n-6 long chain PUFA concentrations (Carlson *et al.*, 1997). Similar concerns about a lack of epidemiological data which support the link between TFAs and increased risk of coronary heart disease in adults have brought the true negative impact of TFAs on human health into question (Shapiro, 1997).

The only fatty acids considered essential for human growth are linoleic (LA) and linolenic (LNA) acid, which are required in a range of 0.5 to 2.0% and 0.5% of total

energy respectively (Bruckner, 1992; Bell *et al.*, 1997). Essential fatty acid deficiency has been associated with growth retardation, increased membrane permeability, sterility and capillary fragility (Vergroesen, 1976). Linoleic acid and LNA are precursors of 20-carbon fatty acids which are precursors of the eicosanoids. Essential fatty acids are particularly important for infants as their levels can have an effect on DHA and AA levels, especially in preterm infants where a high ratio of LA to LNA can reduce the ratio of DHA to AA in the brain and retina (Martinez, 1992; Gibson *et al.*, 1994). Increasing the concentration of LNA in infant formula can also produce an increase in DHA levels (Innis *et al.*, 1997). Competition between LA and LNA for $\Delta6$-desaturase seems to influence the synthesis of both AA and DHA (Innis, 1992). Excessive concentrations of LA in the adult diet can increase the risk of cancer and can predispose membrane phospholipids to free radical oxidation (Grundy, 1997).

The importance of long chain PUFAs such as EPA and DHA in the prevention of heart disease in adults was first realized in the early 1970's (Bang and Dyerberg, 1972). Since then, increased consumption of EPA and DHA, in the form of fish and fish oil capsules has been associated with a reduced risk of atherosclerosis, tumour growth, thrombosis, hypertriglyceridaemia, and high blood pressure (Braden and Carroll, 1986; Shekelle *et al.*, 1981; McGee *et al.*, 1984; Joossens *et al.*, 1989). The inhibitory effect of PUFAs on many of these conditions seems to be related to their mediation of eicosanoid precursor synthesis (Braden and Carroll, 1986). In infants, DHA is required for nervous system and retinal development as shown by reduced data processing time and increased visual acuity in infants fed human milk containing DHA compared to infants fed formula with no added DHA (Makrides *et al.*, 1996; Carlson *et al.*, 1996). An exogenous source of DHA is required by infants because LNA is not readily converted to DHA (Neuringer *et al.*, 1994).

There have been conflicting opinions about the importance of DHA in infant development and consequently about its presence in infant formulas due to conflicting reports about the extent of its effects on visual function in infants. Several authors have found that supplementation of infant formula with DHA has produced some improvement in visual function compared to non-supplemented formula-fed infants for both preterm and term infants, up to one year of age (Werkman and Carlson, 1996; Carlson and Werkman, 1996; Carlson *et al.*, 1993; Makrides *et al.*, 1996). Other authors have found no improvement in visual function, even when comparisons were made between breast-fed, DHA-supplemented and DHA-non-supplemented formula-fed infants (Carlson, 1996; Innis *et al.*, 1996). Preterm infants may have a higher requirement for DHA as well as AA because they are more susceptible to pre- and post-natal deficits of AA and DHA which may lead to neurovisual development disorders (Crawford *et al.*, 1997).

While the traditional source of EPA and DHA has been fish oils, which can contain between 10 and 25% total of these PUFAs (Haraldsson *et al.*, 1993), there is difficulty in supplementing infant formula with this source since EPA seems to compete with AA for incorporation into membranes, resulting in a reduction of membrane AA and a subsequent reduction in the 2-series eicosanoids (Martinez, 1992; Makrides *et al.*, 1995). Feeding infants a fish oil source of DHA in the presence of EPA has been associated with infants with lower normalized weights and lengths than those fed standard infant formula (Carlson *et al.*, 1992). There is controversy regarding the inclusion of EPA in infant formula since it is present in low concentrations (around

0.2% of total fatty acids) in human milk, and its levels can be affected by the mother's diet (Francois *et al.*, 1998). PUFAs in TAGs from algal sources or as phospholipids from eggs are absorbed from infant formula by preterm infants as efficiently as from human milk (Carnielli *et al.*, 1998; Boehm *et al.*, 1997).

The positional distribution of long chain PUFAs, along with chain length and degree of unsaturation are important factors affecting the degree of hydrolysis and absorption. Structured lipids, with the PUFAs in position sn-2 are ideal due to the low activity of pancreatic lipase towards these fatty acids in the sn-1 and sn-3 positions and their improved absorption as 2-MAGs (Christensen *et al.*, 1995). There is some conflict as to the best form of PUFAs for good absorption, although the degree of absorption does not seem to differ (Linko and Hayakawa, 1996; Krokan and coworkers, 1983). Nelson and Ackman (1988) found that ethyl esters of EPA are absorbed better in humans than free fatty acid forms or 2-MAG forms of EPA, while Linko and Hayakawa (1996) found that the degree of absorption of free DHA was greater than 95%, while in the TAG and ethyl ester forms, degrees of absorption were only 57% and 21%, respectively. The advantage of consuming ethyl esters over natural TAGs is that the target fatty acid can be concentrated to a greater extent during processing.

As mentioned previously, long chain PUFAs are important due to their relationship to eicosanoids such as prostaglandins, leukotrienes and thromboxanes, which are derived from three different 20-carbon fatty acids and possess hormone-like activity (Berdanier, 1995). Dihomo-gamma-linolenic acid, arachidonic acid (AA) and EPA are precursors for eicosanoid series 1,2, and 3, respectively, with AA metabolism beginning with LA, and EPA metabolism beginning with α-LNA (*Figure 2*). Methyl-interrupted PUFAs are formed by successive elongation and desaturation, with n-3 PUFAs such as DHA and EPA formed from α-LNA and n-6 PUFAs such as AA formed from LA. While EPA and DHA are produced from LNA, they are still required in the diet because the conversion efficiency of LNA is low, and direct consumption of EPA and DHA is more effective at increasing their levels in plasma lipids (Linko and Hayakawa, 1996). Eicosanoids derived from EPA have relatively weak capabilities (weaker thromboxanes), stimulating and preventing platelet aggregation and causing smooth muscle contraction. Eicosanoids derived from AA have a strong influence, stimulating and preventing aggregation of platelets and contraction of smooth muscle (Gurr, 1992). Replacement of AA with EPA through consumption of fish oils high in EPA inhibits production of strong aggregator thromboxanes by AA, thereby reducing platelet aggregation and reducing the risk of atherosclerosis (Garg *et al.*, 1990). High ratios of LA to saturated fatty acids in the diet also inhibit desaturase activity (Spielmann *et al.*, 1988).

There are some differences in the effects of these precursor fatty acids in infant development. As previously mentioned, the displacement of AA by EPA is not beneficial in infants since it has been associated with reduced growth. Excessive or limited concentrations of LA can also reduce AA levels. The difficulty in predicting the effects of different levels of eicosanoid precursors on health and disease of infants and adults is that each of the three eicosanoid families display both anti-aggregatory and anti-inflammatory precursors. With an understanding of the lipid compositional requirements of different population segments, biotechnology in the form of biotransformations, genetic modification of oilseed crops and lipase-catalysed interesterification have been applied to meet these needs.

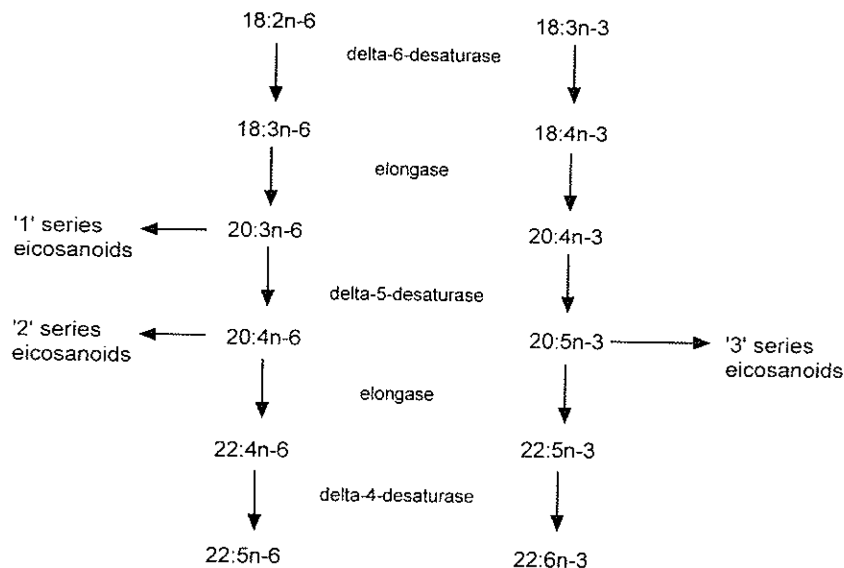

Figure 2. Pathways for production of EPA, DHA (from linolenic acid) and AA (from linoleic acid) and their associated eicosanoids.

Biotransformations: production of PUFAs by microorganisms

SOURCES

With greater demand for high quality and less expensive sources of EPA and DHA for use as supplements in nutritionally improved fats and oils, researchers have turned to microorganisms, mainly algae and fungi, as a major source of PUFAs (*Tables 2,3,4*). The cost of deriving long chain PUFAs from microorganisms has been estimated to be half that of fish oil derived production of PUFAs (Molina Grima *et al.*, 1996). Most long chain PUFAs are derived from eukaryotes such as fungi and algae which produce TAGs in their biomass with fatty acid compositions similar to those of plant oils (Mukherjee, 1998).

Fish oil composition is more variable due to diet-related effects and fish oils are more susceptible to oxidation due to the extended periods between catching and processing, resulting in undesirable flavours and odours (Shimizu *et al.*, 1989). Microbial sources of these PUFAs are superior to fish-derived fatty acids because of improved oxidative stability, the potential to produce high concentrations of specific fatty acids and greater control of fatty acid composition (López Alonso and del Castillo, 1996; Medina *et al.*, 1995; Yongmanitchai and Ward, 1991). It is possible to use microorganisms which produce high concentrations of one specific PUFA, making it easier to isolate this PUFA from other fatty acids (Molina Grima *et al.*, 1996). Other advantages of using microorganisms for PUFA production include low energy costs since operating temperatures are close to room temperature, feasible large scale production, and the potential for the production of value-added by-products (Shimizu *et al.*, 1989; Cohen, 1990).

Table 2. Selected algae, their total lipid content and nutritionally important fatty acids

Organism	Fatty acid of interest	Maximum biomass content (g/L)	Maximum content (% w/w)	Reference
Chlorella minutissima	eicosapentaenoic acid (31.8% of FA)	0.5	14.3 (lipid)	Yongmanitchai and Ward (1991)
Phaeodactylum tricornutum	eicosapentaenoic acid (30.5% of FA)	2.5	17.4 (lipid)	Yongmanitchai and Ward (1991)
Nitzschia laevis	eicosapentaenoic acid (23.2% of FA)	–	39.3 (lipid)	Koon Tan and Johns (1996)
picoplankton strain PP301	eicosapentaenoic acid (53.2% of FA) and docosahexaenoic acid (8.9% of FA)	–	13.6 (FA)	Kawachi *et al.* (1996)
Isochrysis galbana	eicosapentaenoic acid and docosahexaenoic acid (9.5% of FA)	–	9.5 (FA)	Robles Medina *et al.* (1995)
Porphyridium cruentum (113.80)	eicosapentaenoic acid (42% of FA)	–	4.4 (FA)	Cohen (1990)
Phaeodactylum tricornutum	eicosapentaenoic acid (3.1% of biomass, 30.5% of FA)	–	–	Cartens *et al.* (1996)
Isochrysis galbana	docosahexaenoic acid (5.4% of biomass)	0.9	14.8 (FA)	Burgess *et al.* (1993)

Table 3. Selected bacteria, their total lipid content and nutritionally important fatty acids

Organism	Fatty acid(s) of interest	Maximum cell content (g/L)	Maximum content (% w/w)	Reference
Vibrio (strain T3614)	docosahexaenoic (6.7% of FA)	0.1	9.8 (lipid)	Yano *et al.* (1994)
SCRC-2738 (from mackerel intestine)	eicosapentaenoic acid (12.8% of FA)	3.7	5.7 (FA)	Akimoto *et al.* (1990)
SCRC-2738 (from mackerel intestine)	eicosapentaenoic acid (22.5% of FA)	5.3	4.3 (FA)	Akimoto *et al.* (1991)

EXTRACTION AND PURIFICATION

The major difficulty associated with the production of PUFAs using microorganisms centres around the relatively extensive purification process required to isolate and refine the oils. Some methods involve a five-step process using a combination of chloroform, methanol, and water to separate lipid classes followed by transmethylation, urea fractionation and reverse phase chromatography (Cartens *et al.*, 1996). Urea complexation is an effective method of PUFA isolation since urea preferentially complexes with saturated and monounsaturated fatty acids to form solids which can be removed from the PUFAs (Bajpai and Bajpai, 1993). Cartens and coworkers (1996)

Table 4. Selected fungi, their total lipid content and nutritionally important fatty acids

Organism	Fatty acid of interest	Maximum biomass content (g/L)	Maximum lipid content (% w/w)	Reference
Mortierella sp.	g-linolenic (26% of lipid)	11–12	24	Hansson and Dostálek (1988)
Mortierella alpina	arachidonic (31% of lipid)	23	11	Lindberg and Molin (1993)
Mortierella alpina	arachidonic acid (31% of lipid)	22.5	44	Shinmen *et al.* (1989)
Thraustochytrium aureum	docosahexaenoic (40% of lipid)	5.7	8	Iida *et al.* (1996)
Pythium irregulare	eicosapentaenoic acid (25% of lipid)	–	10	O'Brien *et al.* (1993)
Thraustochytrium aureum	eicosapentaenoic (9% of FA) docosahexaenoic (30% of FA)	4	10	Kendrick and Ratledge (1992)
Mortierella alpina-peyron	arachidonic (5% of FA)	3.2	38	Kendrick and Ratledge (1992)
Pythium ultimum	eicosapentaenoic (0.7% of biomass w/w)	3.2	–	Wessinger *et al.* (1990)
Shiizochytrium sp.	docosahexaenoic (34% of FA)	21.0	–	Nakahara *et al.* (1996)
Thraustochytrium aureum	docosahexaenoic acid (5–7% of lipid)	1.1–5.5	1.7–25.2	Bajpai *et al.* (1991)

avoided chloroform and methanol by using ethanolic potassium hydroxide saponification, followed by liquid chromatography to isolate the PUFA fraction. They obtained the same yield of EPA without the urea fractionation step. Medina and coworkers (1995) developed a two-step purification process for algal biomass involving only urea complexation and liquid chromatography, achieving a range of 94–96% purity for fractions of EPA and DHA, respectively. Molina Grima and coworkers (1996) scaled-up recovery of EPA from *P. tricornutum* using a four-step process of fatty acid extraction, saponification, lyophilization, urea fractionation and semi-preparative HPLC.

GROWTH CONDITIONS

Growth of microorganisms is highly dependent on growth media composition including temperature, pH, source, amount and ratio of carbon and nitrogen sources, and the degree of light exposure and aeration (Cohen, 1990; Bajpai and Bajpai, 1993; Rose, 1989). There are major differences in the growth conditions of different organisms, which can affect both the lipid content and fatty acid composition of the biomass (O'Brien *et al.*, 1993; Akimoto *et al.*, 1991).

In general, decreasing the temperature produces an increase in the concentration of unsaturated fatty acids, although a decrease in total lipid in the biomass can also occur (Lindberg and Molin, 1993). Membrane lipids, and in particular, phospholipids must remain in a liquid-crystalline state at lower temperatures to allow for normal membrane

activity. This requires an increase in the degree of unsaturation of their component fatty acids at lower temperatures. Several authors have verified that this increase in the concentration of unsaturated fatty acids at lower temperatures occurs in both algae and fungi (Hansson and Dostálek, 1988; Lindberg and Molin, 1993; Burgess *et al.*, 1993). As previously mentioned, the optimum temperature for growth of both fungi and algae is usually between 20 and 30°C, with the temperature being modified to maximize concentrations of specific PUFAs (Kawachi *et al.*, 1996; Burgess *et al.*, 1993; Akimoto *et al.*, 1990). This temperature range provides an economical means for scale-up and production and allows production both indoors and outdoors. Molina Grima and coworkers (1995) were able to produce *I. galbana* outdoors in Spain and found that productivity was greatest in the early part of the summer when the average outdoor temperature brought the culture temperature closest to its optimum growth temperature of 20°C. Optimum pH values for individual organisms vary, although most tend to be in the range of pH 5.5 to 8 (Lindberg and Molin, 1993; Iida *et al.*, 1996).

Production of PUFAs is highly dependent on the growth stage of the microorganism. While there is generally rapid production of PUFAs during the growth phase of the organisms, concentrations can be dramatically increased by ageing at steady state for several days at lower temperatures (Bajpai and Bajpai, 1993; Akimoto *et al.*, 1990; Shimizu *et al.*, 1989; Bajpai *et al.*, 1991). The fatty acid composition can also be affected by growth rate and storage time. Cohen (1990) found that the AA concentration produced by the microalga, *Porphyridium cruentum*, could be increased by nitrogen starvation and decreased growth, while with rapid growth, the concentration of EPA increased. Burgess and coworkers (1993) found that there was increased production of DHA by *I. galbana* by using storage conditions of low temperature and low light after the growth phase.

All organisms require a source of carbon and nitrogen for growth, although the sources can vary widely. Bajpai and coworkers (1991) found that the fungi *Thraustochytrium aureum* grew best on a carbon source of linseed oil, glucose or starch, while O'Brien and coworkers (1993) were able to use sweet whey permeate as a lactose source for fungal growth. The presence of vegetable oils such as soybean, corn, peanut and rapeseed oil, which have a high concentration of oleic and linoleic acid seems to promote the production of EPA and DHA (Shinmen *et al.*, 1989). EPA and DHA are produced from monounsaturated fatty acids, such as oleic acid which are desaturated and elongated via the n-3 pathway to produce LA then EPA and DHA, or the n-6 to produce LNA and AA (Bajpai and Bajpai, 1993). Kanisaka and coworkers (1990) also found that oleic and linoleic acid were both used rapidly by *Mortierella* sp. and desaturated to γ-linolenic acid during its growth phase. Sources of nitrogen may include potassium nitrate, corn steep liquor or yeast extract (Shinmen *et al.*, 1989; Hansson and Dostálek, 1988; Nakahara *et al.*, 1996). The concentration of nitrogen has an impact on the composition of fatty acids in the lipid fraction as shown by Cohen (1990), who found that starving the red microalga *Porhpyridium cruentum* produced a sharp increase in the concentration of AA. The ratio of carbon to nitrogen also seems to be important. Hansson and Dostálek (1988) found that a ratio of carbon to nitrogen of 80 produced the highest lipid concentration of 66% (w/w) in the biomass.

The fatty acid composition of light sensitive microorganisms can vary significantly during periods of light and dark. Storage fatty acids such as palmitic and palmitoleic

acids in *I. galbana* accumulate in the presence of light and are used for energy in the absence of light (Molina Grima *et al.*, 1995). Bajpai and coworkers (1991) found that increasing the time of light exposure caused the fungi, *Thraustochytrium aureum*, to increase production of DHA, while Burgess and coworkers (1993) found that reducing light exposure caused the algae, *Isochrysis galbana*, to increase production of DHA. Photosynthetic organisms such as marine algae are superior to microbial cells because they can use sunlight for energy and CO_2 as a carbon source (Burgess *et al.*, 1993). Photosynthetic organisms also have increased concentrations of unsaturated fatty acids compared to heterotrophic organisms (Koon Tan and Johns, 1996).

SCALE-UP AND INCREASING PRODUCTIVITY

It can be difficult to scale up fungal and algal growth without experiencing difficulties. Using fungi for production has some inherent problems, including low growth rates, high viscosity and adhesion to surfaces (Hansson and Dostálek, 1988). Microalga are superior in this respect because they tend to autoflocculate, improving the harvest process (Cohen, 1990).

Fungi grow very well on solid media, but are much easier to grow in liquid media on a large scale, usually in shaker flasks (Shinmen *et al.*, 1989; Lindberg and Molin, 1993). Iida and coworkers (1996) found that a flask culture was superior to a fermenter culture of *T. aureum*, due to inhibition of growth from the stirring which occurred in the fermenter culture. Fukuda and Morikawa (1987) were able to improve the growth of fungi in fermenter cultures by immobilizing the cells with biomass support particles and by using a fluidized bed for mixing.

More recent efforts at improving the productivity of microorganisms have been concerned with designing equipment for scale up and controlling the growth environment as much as possible. As well, genetic modification has become another avenue to increase productivity of specific organisms (López Alonso and del Castillo, 1996). Productivity in terms of the total amount of specific fatty acids produced as opposed to the highest percentage of the total lipid content must be considered.

Genetic modification of oilseed crops

Genetic modification of oilseed crops to improve quality, pest and disease resistance and yield has expanded in recent years to include modification of the fatty acid composition of oils for food use as well as for oils used as lubricants and detergents (Murphy, 1996).

In general, oilseeds tend to accumulate LCFAs containing 16 or 18 carbons with one to several double bonds (Ohlrogge, 1994). Long chain fatty acid synthesis occurs in the plastids by sequential addition of two carbon units from acetyl-CoA to an acyl carrier protein (ACP) to eventually produce palmitoyl-acyl-carrier protein (palmitoyl-ACP) which is then removed from the ACP by a thioesterase (*Figure 3*). Elongation and desaturation of the fatty acid occurs in the cytoplasm to form other fatty acids (Murphy, 1994; Miquel and Browse, 1995; Slabas *et al.*, 1992). Triacylglycerols are formed by the sequential acylation of glycerol-3-phosphate to first form 1-acyl-*sn*-glycerol-3-phosphate then phosphatidic acid by lysophosphatidic acid acyltransferase. The phosphatidic acid is eventually converted to a diacylglycerol and then a

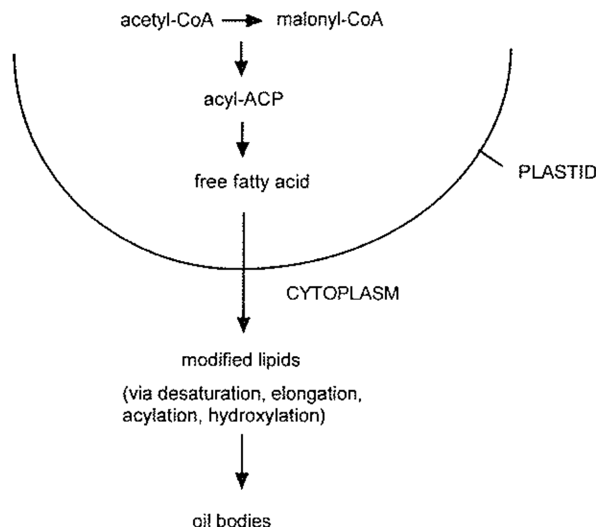

Figure 3. Simplified pathway for biosynthesis of fatty acids in plants (adapted from Jaworski *et al.*, 1992 and Slabas *et al.*, 1992).

triacylglycerol with the addition of another acyl group (Yuan and Knauf, 1998). The positional distribution of fatty acids in the triacylglycerol can be modified by the activity of lysophosphatidic acyltransferases from different sources which are specific towards fatty acids of differing chain lengths, such as for lauric acid by this acyltransferase in canola oil (Knutzon *et al.*, 1995).

The main method of fatty acid profile modification is the cloning and transfer of a gene from one plant species into another species to produce the desired levels of specific fatty acids. As well, naturally occurring enzymes can be modified or new ones can be introduced to modify the fatty acid profile of the oilseed. Once an enzyme has been identified and isolated, its associated gene can be identified and modified (Del Vecchio, 1996). Genes from bacterial, animal and yeast sources have also been incorporated into oilseeds for fatty acid modification (Miquel and Browse, 1995). Yadav and coworkers (1992) used a T-DNA tagging method to isolate the *Arabidopsis* microsomal n-3 fatty acid desaturase responsible for LNA to convert more than 75% of the LA in the seeds into LNA. A gene encoding lauric acid production from the California bay (*Umbellularia californica*) has been incorporated into *Arabidopsis thaliana*, resulting in a 70 fold increase in the 12:0-ACP thioesterase activity, and a subsequent increase in the concentration of lauric acid (Voelker *et al.*, 1992).

Previously, the main focus of transgenic modifications had been in the reduction of erucic acid in rapeseed oil. Since then, the focus has shifted towards encouraging production of medium chain fatty acids, including high lauric acid concentrations in canola oil (Del Vecchio, 1996). Some plant species produce high concentrations of MCFAs by a mechanism which seems to involve premature chain termination by acyl-ACP thioesterase to produce chain lengths of eight to fourteen carbons (Slabas *et al.*, 1992; Davies *et al.*, 1993). It is possible to further increase the medium chain fatty acid composition of oils such as the high laurate canola oil by cloning a gene which encodes for an acyltransferase which is specific for medium chain fatty acids (Knutzon *et al.*,

1995). While some seed oils, such as palm kernel oil, are not modified because they naturally contain high concentrations of MCFAs, other plants, including *Cuphea* species have been engineered to increase their medium chain fatty acid concentration (Wiberg and Bafor, 1995; Knapp *et al.*, 1991). Knapp and coworkers (1991) found that in *Cuphea viscosissima,* the total medium chain fatty acid concentration could be increased above an already high level of approximately 88% by mutational modifications. Davies and coworkers (1993) studied the ACP-thioesterases from different plant species and found that they had differing medium chain fatty acid specificities which correlated well with specific fatty acid composition in the seed. Dehesh and coworkers (1996) were able to redirect fatty acid synthesis in canola oil by performing a transgenic expression of a 8:0/10:0 specific thioesterase from *Cuphea hookeriana* to incorporate 8:0 and 10:0 into canola oil.

The disadvantages associated with genetic engineering of oilseed crops are mainly related to a lack of understanding and characterization of the processes involved in the fatty acid modification of oilseeds. Gene cloning is time consuming and expensive and there is a lack of understanding of the full implications of genetic modification on the biochemistry and regulation of oilseed metabolic processes (Miquel and Browse, 1994). The practical problems associated with crop management include pest and disease resistance and prevention of cross-pollination by non-modified plants, making the process economically unfeasible. At the present time, there are a limited number of available sequence encoding enzymes which are key in lipid metabolism, which slows down the process of gene isolation (Wolter, 1993). Despite these problems, there are several advantages associated with modifying the fatty acid profile at the level of growth and development of oilseed crops. In general, during incorporation of MCFAs into TAGs in the oilseed, there is a natural tendency for the fatty acid composition at the sn-2 position to be maintained as a long chain unsaturated fatty acid, with the MCFAs occupying positions 1 and 3 (Del Vecchio, 1996). This provides a structured lipid, which at the moment is really only obtainable at the present time by lipase-catalysed interesterification. As well, work is also being performed to encourage production of PUFAs in oilseeds, with some success already accomplished with increasing the concentration of γ-LNA through expression of the Δ6-desaturase gene (Reddy and Thomas, 1996). The ultimate goal in this case is to produce EPA and DHA in oilseeds.

Lipase-catalysed modification of fats and oils

METHODS

Traditionally, chemical interesterification has been the primary industrial means of producing modified fats and oils. It is an entropically driven reaction which, in theory, produces a complete randomization of acyl groups in TAGs (Coenen, 1974; Kuksis *et al.*, 1963; Ferrari *et al.*, 1997). Chemical interesterification is used in the manufacture of low-*trans* shortenings, margarines and spreads to improve their textural properties, modify melting behaviour and enhance stability (Nawar, 1996; Ghazali *et al.*, 1995). Chemical interesterification has the potential to be applied to the nutritional improvement of fats and oils, mainly to increase the proportion of specific fatty acids in specific positions on the glycerol backbone to improve their bioavailability. Chemical

interesterification is not an effective method of producing high concentrations of MCFAs, nor structured lipids due to the randomness and lack of positional or fatty acid selectivity inherent in this method (Klemann *et al.*, 1994; Ray and Bhattacharyya, 1995). Lipase-catalysed interesterification is superior to chemical interesterification when specific positional distributions are required due to the inherent positional and fatty acid specificity possessed by lipases. Lipases catalyse the hydrolysis of TAGs, DAGs and MAGs in the presence of excess water, but under water limiting conditions, the reverse reaction, ester synthesis, can be achieved (Macrae, 1985; Jaeger *et al.*, 1994). Lipase-catalysed interesterification and hydrolysis follow a Ping Pong Bi Bi reaction for multisubstrate reactions (Malcata *et al.*, 1992; Reyes and Hill Jr, 1994).

In terms of the application of lipases to the nutritional modification of fats and oils, both the positional and fatty acid specificity of certain lipases are used (*Table 5*). Lipases which possess no specificity produce the same positional distribution as chemical interesterification with significantly greater cost and time requirements, making them unsuitable for this application (Macrae, 1983, Gunstone, 1994). Positional specificity towards positions 1 and 3 of the TAG is due to an inability of the lipase to act on position sn-2 because steric hindrance prevents access of the fatty acid in the sn-2 position to the active site (Macrae, 1983; Macrae and How, 1988). Fatty acid specificity, in terms of specificity both towards and against different fatty acids has been employed in the removal or concentrations of these fatty acids. The positional and fatty acid specificity of different lipases has been used in numerous lipase-catalysed reactions, including transesterification, acidolysis, glycerolysis and esterification to improve the nutritional quality of fats and oils. Lipases have also been used in the modification of fats and oils for the purpose of physical modifications, for use as cocoa butter substitutes and to alter melting properties.

Table 5. Fatty acid and positional specificity of selected lipases used in the nutritional modification of fats and oils

Source of lipase	Specificity	Reference
Aspergillus niger *Aspergillus delemar*	Towards medium and short chain fatty acids	Desnuelle (1972); Stamatis *et al.* (1993)
Geotrichum candidum	Towards long chain fatty acids with *cis*-9 double bonds	Macrae (1985)
Aspergillus niger *Mucor miehei* *Rhizopus arrhizus* *Rhizopus delemar*	Towards positions sn-1 and sn-3	Macrae (1983)
Candida parapsilosis	Towards sn-2 position	Riaublanc *et al.* (1993)

Transesterification

Lipase-catalysed transesterification is defined as the exchange of acyl groups between two esters, namely two TAGs, although it can also be between ethyl or methyl esters and TAGs (*Figure 4*). Transesterification is not used as a method to transfer PUFAs from fish oils to vegetable oils due to the relatively low concentration of EPA and DHA in fish oils which does not usually exceed 25% (Ackman, 1988). It is most commonly used to produce structured lipids by the reaction between a medium chain

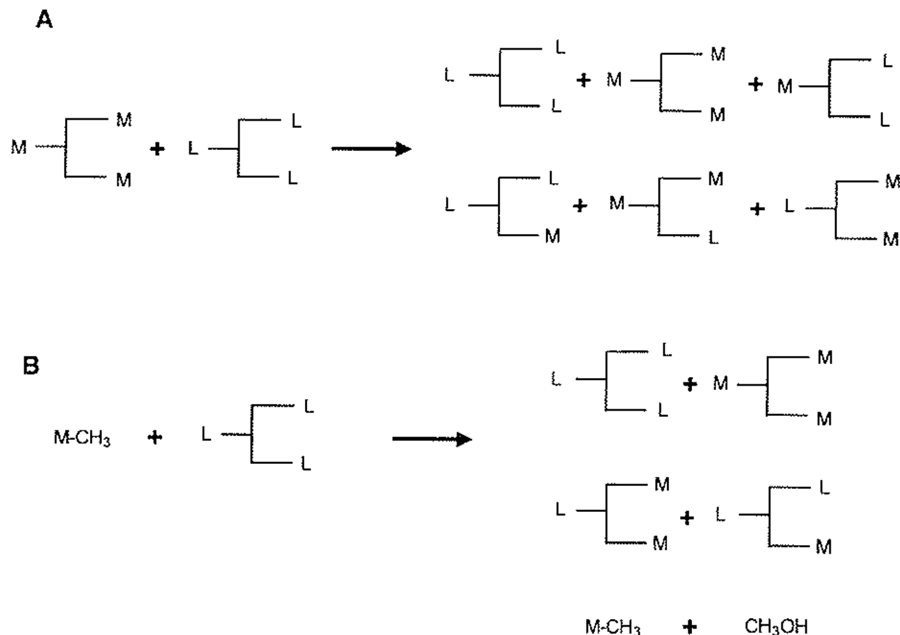

Figure 4. Possible TAG species derived from a 1,3-specific lipase-catalysed transesterification reaction between a medium chain TAG (A) or medium chain methyl ester (B) and a long chain TAG.

triacylglycerol and a vegetable oil or fish oil containing high concentrations of long chain essential and polyunsaturated fatty acids. This is not considered the ideal method for structured lipid production because there is a randomization of medium, essential and polyunsaturated fatty acids in all three positions of the triacylglycerol. Structured lipids should ideally contain medium chain fatty acids in positions sn-1 and sn-3 for rapid hydrolysis and absorption for energy production, and long chain essential and polyunsaturated fatty acids in the sn-2 position to improve their absorption. Higher concentrations of structured lipids are more easily obtained using acidolysis reactions.

Acidolysis

Acidolysis is defined as the transfer of an acyl group between an acid and an ester, and is used mainly to incorporate novel FFAs into TAGs (*Figure 5*). Acidolysis between a PUFA-rich fraction and fish oils has been a successful way of increasing the PUFA concentration in fish oils, since fish oils tend to have PUFAs in the sn-2 position, allowing more to be incorporated into positions sn-1 and sn-3.

Polyunsaturated fatty acid-enriched fish oils have been used in encapsulated form to reduce the risk of cardiovascular disease in adults. These oils are not suitable for use in infant formulas due to the high concentration of EPA which may compete with AA and affect growth. PUFA-enriched vegetable oils have been used in the prevention of cardiovascular disease in adults. The advantage of acidolysis over transesterification to produce structured lipids is a greater degree of incorporation. However, there are still difficulties in placing PUFAs in position sn-2 and MCFAs in positions 1 and 3.

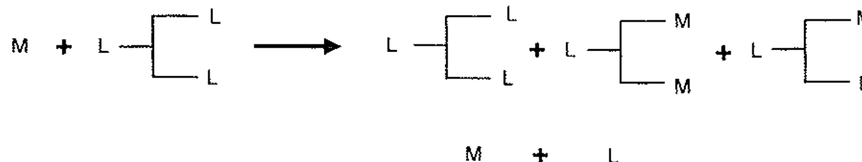

Figure 5. Potential TAG species derived from a 1,3-specific lipase-catalysed acidolysis reaction between a long chain TAG and a medium chain fatty acid.

There are major disadvantages associated with using acidolysis as a means of improving the nutritional quality of fats and oils. Obtaining a fatty acid concentrate with high concentrations of DHA and/or EPA requires several steps, including saponification, solvent extraction, and urea inclusion or molecular distillation (Li and Ward, 1993a). Since fatty acids from the original TAG are released during the course of acidolysis, those fatty acids plus those remaining from the original substrate must be removed from the lipid. Due to the heat lability of PUFAs, traditional means of fatty acid removal such as molecular distillation have been replaced by methods such as titration with salts to precipitate fatty acids (Tanaka *et al.*, 1994).

Glycerolysis/esterification

Glycerolysis is the reaction between a TAG and glycerol, while esterification is the reaction between glycerol (or an alcohol group such as a partial glyceride) and a free fatty acid (*Figure 6*). The main application of esterification is in the production of TAGs containing all long chain PUFAs for use as adult supplements or all MCFAs for parenteral nutrition, while glycerolysis has been used to produce PUFA-containing MAGs. The advantage of glycerolysis and esterification is the high purity of TAGs containing only one fatty acid type which can be obtained, although the yield of TAGs tends to be low. The disadvantage of esterification is that the fatty acid substrates remaining must be removed. As well, esterification using ethyl esters remains economically unfeasible due to the high production cost of producing EPA and DHA concentrates (Li and Ward, 1993a; Sridhar and Lakshminarayana, 1992).

Hydrolysis/selective enrichment

As mentioned previously, some lipases are specific towards certain fatty acids, a property which can be used to concentrate these fatty acids during hydrolysis and transesterification. Lipases with decreased specificity towards DHA have been used frequently to increase the concentration of DHA in fish oil. The lower activity of some lipases towards DHA is attributed to the fact that the carbon-carbon double bond nearest to the carboxyl group is one carbon closer in DHA than in EPA which affects its ability to fit into the active site (Haraldsson *et al.*, 1993). While this selective enrichment has been used effectively in the nutritional modification of fats and oils, care must be taken to recognize the specificities of these lipases when conducting other lipase-catalysed modification methods to prevent low levels of incorporation of some fatty acids (Kosugi and Azuma, 1994; Haraldsson *et al.*, 1993; Langholz *et al*, 1989). Overall, selective enrichment is a promising method for the concentration of fatty

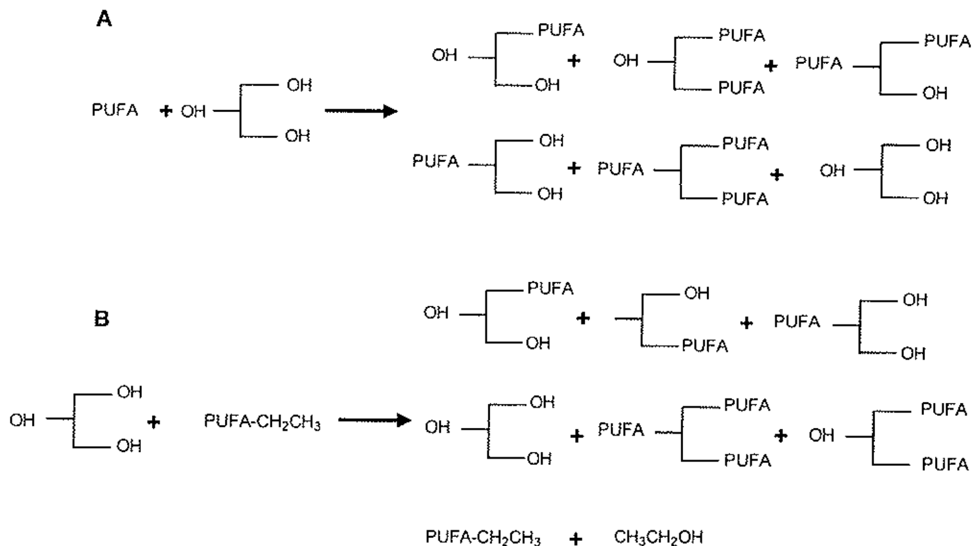

Figure 6. Potential TAG species from a non-specific lipase-catalysed esterification (A) and glycerolysis (B) reactions between glycerol and a PUFA and glycerol and a PUFA-ethyl ester, respectively.

acids in oils, specifically fish oils, since it does not require PUFA concentrates which are difficult and expensive to manufacture.

PRODUCTS

In terms of the nutritional modification of lipids, most of the lipase-catalysed methods described previously have been used to produce varied products, including medium chain triacylglycerols, structured lipids, PUFA concentrated fish oils, and PUFA containing vegetable oils. Some methods are superior for the production of specific products. As well, lipase-catalysed interesterification has been used to produce cocoa-butter substitutes and to modify the physical properties of certain lipids.

Medium chain triacylglycerols

Medium chain triacylglycerols are used predominantly in the manufacture of parenteral and enteral formulations, and in infant formulas for preterm infants since medium chain triacylglycerols are more rapidly hydrolysed and metabolised compared to long chain triacylglycerols (Mascioli *et al.*, 1988). Medium chain triacylglycerols have been blended with long chain triacylglycerols in these formulations to reduce the added cost associated with structured lipid production, although there are differing opinions as to which method is more nutritionally beneficial (Sobrado *et al.*, 1985; Christensen and Høy, 1997).

In order to produce pure medium chain triacylglycerols, esterification of glycerol and medium chain fatty acids has been the lipase-catalysed method of choice (*Table 6*). Kwon and coworkers (1996) produced MCTs from esterification of glycerol with capric acid, to obtain 47.6% (w/w) tricaprylin after 24 hours. However, lipases are not

Table 6. Production of medium-chain triacylglycerols and concentration of medium chain fatty acids by lipase-catalysed interesterification reactions

Source of lipase	Method	Substrate	Yield	Reference
Candida rugosa	esterification	capric acid glycerol	56.2 mol% tricaprin of all caprin-containing species	Kwon *et al.* (1996)
Mucor miehei	acidolysis	capric acid caprylic acid methyl esters coconut oil	capric acid increased from 6.2% to 18% carpylic acid increased from 4.9% to 17.9% (w/w)	Ghosh and Bhattacharyya (1997)

usually used to produce medium chain triacylglycerols since chemical esterification and fractionation are more cost effective. The major use of medium chain triacylglycerols is in the production of structured lipids, using 1,3-specific lipases.

Structured lipids

As previously defined, structured lipids (*Table 7*) may contain a variety of medium chain, essential and polyunsaturated fatty acids, preferably with the medium chain fatty acids in positions sn-1 and sn-3 and the long chain fatty acids in the sn-2 position.

Using a 1,3-specific lipase, acidolysis is superior to transesterification because it allows placement of medium chain fatty acids specifically in positions sn-1 and sn-3. Transesterification produces a mixture of TAGs because the starting species are both TAGs, one containing medium chain fatty acids and the other containing essential or polyunsaturated fatty acids in positions sn-1 and sn-3. Lee and Akoh (1996) transesterified tricaprin and triolein and found that the final product contained 43.3–57.7% capric acid and 42.3–56.4% LA in the sn-2 position. As well, while Soumanou and coworkers (1997) were able to produce 73%(w/w) structured lipids from transesterification, only 31% of the TAGs were disubstituted.

Despite the greater control of final product composition attainable by acidolysis, acidolysis still requires removal of residual fatty acid substrate and fatty acids produced during the reaction. As well, acyl migration may occur, resulting in placement of MCFAs in the sn-2 position. For this reason, transesterification still seems to be the reaction of choice for producing structured lipids. However, Shimada and coworkers (1996) were able to produce significant improvements in yields by running an acidolysis reaction repeatedly, for a total of three runs, to obtain a final vegetable oil product containing 100% caprylic acid in positions sn-1 and sn-3. Since this yield is significantly higher than any achievable by transesterification, the added cost of removing residual fatty acids from the acidolysis may be justified, particularly in the use of structured lipids in parenteral and enteral formulations where a high degree of substitution would be beneficial.

PUFA concentration in and from fish oils

The greatest interest in the nutritional modification of lipids in recent years has been related to increasing the n-3 PUFA concentration of fish oils above their natural

Table 7. Production of structured lipids by lipase-catalysed interesterification reactions

Source of lipase	Method	Substrates	Yield	Reference
Rhizomucor miehei	transesterification	tricaprylin peanut oil	79 mol% SL	Soumanou *et al.* (1997)
Chromobacterium viscosum			71 mol% SL	
Candida sp.			68 mol% SL	
Mucor miehei *Candida antarctica*	transesterification tricaprin	trilinolein	78 mol% SL	Lee and Akoh (1997)
Mucor miehei	transesterification	tricaprylin EPA ethyl esters	34 mol% EPA incorporated	Lee and Akoh (1996)
Rhizopus delemar	repeated acidolysis	safflower, linseed oil caprylic acid	100% SL	Shimada *et al.* (1996)
Rhizomucor miehei	transesterification	triolein	87.7 mol%	Huang and
		caprylic acid ethyl esters	SL	Akoh (1996)
Mucor miehei	acidolysis	tricaprylin EPA	62% (w/w) EPA incorporated	Shishikura *et al.* (1994)

content of 25% EPA and DHA, because of the reduced risk of atherosclerosis related to consuming higher concentrations of EPA and DHA. Increasing concentrations of EPA and DHA has been achieved through modification of existing fish oils and through production of TAGs containing only EPA or DHA (*Table 8*). Lipases are ideal for modifying fish oils because other physical methods such as winterization, solvent crystallization and molecular distillation are not applicable due to a wide variation in fatty acid composition in the oils. Selective enrichment, through hydrolysis using lipases which are specific against DHA or EPA is a less expensive lipase-catalysed method of increasing the n-3 PUFA content in fish oils because it does not require an external source of concentrated DHA and EPA (*Table 9*). Lipases from *Mucor miehei* and *Candida cylindraceae* are specific against DHA, allowing enrichment of this fatty acid. Hills and coworkers (1990) were able to enrich DHA in cod liver oil from 9.4% to 45%, while Tanaka and coworkers (1992) were able to enrich the DHA content of tuna oil from 25.1% to 53% (w/w). Selective enrichment has also been applied to the enrichment of both EPA and DHA using lipase from *Geotrichum candidum* (Shimada *et al.*, 1994). Docosahexaenoic acid has also been concentrated in the FFA fraction by selective esterification between an alcohol such as butanol or lauryl alcohol and partial glycerides, with fatty acids other than DHA being esterified to the alcohol (Shimada *et al.*, 1997; Hills *et al.*, 1990). The main disadvantage of using this method to concentrate n-3 PUFAs in fish oils is that the fatty acids are concentrated in the form of MAGs and DAGs as a result of the selective hydrolysis process.

To avoid the production of MAGs and DAGs during enrichment, acidolysis is the only other method that has been used to modify fish oil. Adachi and coworkers (1993) used a 1,3-specific lipoprotein lipase from *Pseudomonas* sp. to enrich the total EPA and DHA concentration in sardine oil from 29% to 44.5% (w/w). A combination of acidolysis and low temperature crystallization was used by Yamane and coworkers (1993) to remove saturated and monoenoic fatty acids from the reaction mixture and

Table 8. Production of high concentrations of EPA and DHA-TAGs and concentration of EPA and DHA in fish/whale oil by lipase-catalysed interesterification reactions

Source of lipase	Method	Substrates	Yield (% w/w)	Reference
Pseudomonas sp.	glycerolysis	seal oil whale oil glycerol	42–53% MAGs	Myrnes *et al.* (1995)
Candida cylindraceae	esterification	EPA and DHA other PUFAs (18:1–18:4) glycerol	18–33% TAGs	Osada *et al.* (1990)
Candida antarctica	esterification	EPA and DHA glycerol	100% Tri-EPA and Tri-DHA	Haraldsson *et al.* (1993)
Pseudomonas sp.	esterification	EPA and DHA in a cod liver oil fatty acid concentrate glycerol	18.1% TAGs containing 36.7%	Li and Ward (1993b)
Candida antarctica	glycerolysis	EPA and DHA ethyl esters glycerol	95% Tri-DHA	Kosugi and Azuma (1994)
Mucor miehei	acidolysis	EPA, DHA enriched FA fraction cod liver oil	increased EPA and DHA content by 10%	Yamane *et al.* (1993)
Candida cylindraceae *Chromobacterium viscosum* *Pseudomonas sp.*	acidolysis	EPA, DHA enriched FA fraction sardine oil	65% EPA and DHA in oil	Adachi *et al.* (1993)
Candida antarctica	esterification	EPA glycerol	99% Tri-EPA	Haraldsson *et al.* (1995)

push the reaction equilibrium towards increased PUFA incorporation. Performing acidolysis to increase DHA concentrations in fish oil can be difficult, as found by Tanaka and coworkers (1994), who found that DHA was actually removed from a partially hydrolysed tuna oil fraction to increase the concentration of DHA in the free fatty acid fraction from 13% to 55%, thereby reducing the concentration of DHA in the actual oil. This transfer was due to the low initial concentration of DHA in the free fatty acid fraction.

Esterification between glycerol and n-3 PUFAs is an alternative method for obtaining highly concentrated sources of DHA and EPA. This method allows the production of high purity pure fractions of TAGs containing DHA and EPA. The main disadvantages of this method are the cost of obtaining a relatively pure free fatty acid substrate and the low yields of TAGs which are obtained. Kosugi and Azuma (1994) performed a batch reaction using free DHA and glycerol producing purified 95% TAG, but with yields of 66.2% DHA and 60.1% EPA. Lipases which are 1,3-specific can still be used in esterification reactions, as shown by Li and Ward (1993b) who found that lipase from *Mucor miehei* (Lipozyme IM 60) produced 92% TAGs from a PUFA concentrate and glycerol, while a non-specific lipase from *Pseudomonas* sp. (PS 30) only produced 82% TAGs. Polymerization of EPA and DHA during esterification

Table 9. PUFA concentration in fish oil by selective hydrolysis and enrichment

Source of lipase	Target fatty acid	Oil	Yield (% w/w)	Reference
Geotrichum candidum	AA, EPA, DHA	tuna oil	85.5% TAG, with 81.5% recovery of DHA and EPA	Shimada *et al.* (1994)
Mucor miehei	DHA	cod liver oil	increase of DHA from 9.4% to 45%	Hills *et al.* (1990)
Candida cylindraceae	DHA	tuna oil	46.2% DHA in TAG fraction	Tanaka *et al.* (1992)
Rhizopus niveus	DHA	cod liver oil	29.2% DHA in MAG, 15.2% in TAG (9.6% initially)	Yadwad *et al.* (1991)
Candida rugosa *Geotrichum candidum*	DHA DHA, EPA	cod liver oil	increased EPA and DHA from 30 to 45% in partial glycerides	McNeill *et al.* (1996)
Candida cylindraceae	DHA, EPA	cod liver oil, sardine oil	50% in partial glycerides	Hoshino *et al.* (1990)
Chromobacterium viscosum	DHA	tuna oil	46.2% DHA in TAG fraction	Tanaka *et al.* (1994)
Rhizopus arrhizus	DHA	tuna oil	89% DHA in FFA fraction	Shimada *et al.* (1997)

has also been reported, requiring the addition of higher concentrations of free fatty acids or the use of the ethyl ester form of the fatty acids which do not readily polymerize, although there seems to be a lower degree of incorporation when using the ethyl ester forms (Kosugi and Azuma, 1994; Bech Pedersen and Holmer, 1995).

As of yet, any of these modifications to increase the n-3 PUFA concentration in fish oils or to produce TAGs containing only these fatty acids have had limited applications to making products geared towards disease prevention in adults. Flavour and odour problems associated with high oxidative susceptibility of PUFAs and fish oils limits their use to encapsulated oil products.

PUFA incorporation into vegetable oils

In order to increase the availability of n-3 PUFAs, acidolysis has been used to incorporate them into vegetable oils (*Table 10*), and some degree of success has been achieved. Sridhar and Lakshminarayana (1992) were able to modify the composition of groundnut oil, a staple oil in India by incorporation of a total of 17.5% EPA and DHA into positions 1 and 3. As well, Li and Ward (1993a) were able to incorporate a total of 17.7% EPA and DHA into corn oil. However, in both of these experiments, a 1,3-specific lipase was used, placing these fatty acids in positions sn-1 and sn-3. Pancreatic lipase, shows little activity towards these fatty acids so their potential for hydrolysis and absorption is reduced. Even with acyl migration or using a non-specific lipase to obtain some n-3 PUFAs in the sn-2 position, a larger proportion of these fatty acids will remain in positions sn-1 and sn-3. Therefore, while incorporating EPA and

Table 10. Incorporation of EPA and DHA into vegetable oil by lipase-catalysed interesterification reactions

Source of lipase	Method	Substrate	Yield (% w/w)	Reference
Mucor miehei *Candida antarctica*	acidolysis	EPA, EPA ethyl esters canola oil	18% EPA or 32.9% EPA ethyl esters (mol%)	Huang and Akoh (1994)
Mucor miehei	acidolysis	EPA, DHA corn oil	17.7% (W/w) EPA and DHA	Li and Ward (1993a)
Candida antarctica	acidolysis	EPA evening primrose oil	43% (mol%) EPA	Akoh *et al.* (1996)
Mucor miehei	acidolysis	EPA and DHA ground nut oil	9.5% EPA and 8.0% DHA (w/w)	Sridhar and Lakshmina-rayana (1992)
Candida antarctica	transesterification	EPA ethyl esters trilinolein	81.4 mol% EPA	Akoh *et al.* (1995)
Candida cylindraceae	selective enrichment	*B. orientalis* seed oil	up to 41% (w/w) 20:3 and 20:4 in partial glyceride fraction	Lie Ken Jie and Rahmstullah (1995)

DHA into vegetable oils would increase their use in the food industry, their concentration and availability in these oils remains low.

MODIFICATION OF PHYSICAL PROPERTIES

While the major focus of biotechnology has been on the nutritional modification of lipids, lipases have also been used in the modification of physical properties in the production of cocoa butter equivalents and modification of melting properties.

Cocoa butter equivalents

The high cost of cocoa butter for use in the confectionery industry compared to the relatively low cost of other fats and oils containing similar concentrations of the same fatty acids has resulted in attempts to manufacture cocoa butter equivalents using lipases. Cocoa butter contains 1-palmitoyl-2-oleoyl-3-stearoyl-glycerol (POS) and 1,3-distearoyl-2-oleoyl-glycerol (SOS) as its major triacylglycerol species, representing about 70% of the total (Chang *et al.*, 1990). Several inexpensive sources of lipid have been used, including palm oil midfraction or shea oil combined with stearic acid, hydrogenated cottonseed oil and olive oil, and kokum fat and palmitic acid methyl esters (Macrae and How, 1988; Sridhar *et al.*, 1991; Chang *et al.*, 1990; Bloomer *et al.*, 1990). Since stearic acid is required only in positions 1 and 3 of the triacylglycerol, a 1,3-specific lipase is ideal. Stearic acid or methyl or ethyl stearate are added by acidolysis or transesterification (respectively) to produce a combination of POS, SOS and POP. Several studies have been performed to produce cocoa butter equivalents using lipase-catalysed interesterification (*Table 11*). Macrae and How (1988) developed 1,3-specific-lipase-catalysed acidolysis processes using palm oil liquid fractions, shea oil and stearic acid to produce lipid products with compositions similar to that of

Table 11. Production of cocoa butter equivalents using lipases

Source of lipase	Method	Substrates	Effectiveness	Reference
Rhizopus arrhizus	acidolysis	palm oil midfraction stearic acid	similar concentration of stearic, oleic, palmitic acid	Mojovic *et al.* (1993)
IM 20	acidolysis	palm olein	39.3% (w/w) cocoa butter-like TAGs	Chong *et al.* (1992)
Mucor miehei	transesterification	kokum fat methyl palmitate methyl stearate	peak melting point 32.8°C vs 32.7°C for cocoa butter	Sridhar *et al.* (1991)
Mucor miehei	transesterification	cottonseed oil olive oil	19% (2/2) cocoa butter-like TAGs	Chang *et al.* (1990)

cocoa butter. Chang and coworkers (1990) transesterified hydrogenated cottonseed oil and olive oil to obtain a mixture of POP, OSO, POS and SOS, resulting in a yield of 19% cocoa butter-like fat with a melting range of 29 to 49°C compared to a range of 29 to 43°C for cocoa butter. The cocoa butter fraction was isolated from the other fat by crystallization in acetone. Similarly, Chong and coworkers (1992) ran an acidolysis reaction between stearic acid and palm olein, and obtained a higher yield of cocoa butter-like triacylglycerols. They used steam distillation to remove free fatty acids and fractional crystallization with hexane or acetone to isolate the cocoa butter-like triacylglycerols.

The main consideration associated with using lipase-catalysed interesterification to produce cocoa butter equivalents is keeping the cost low. Major considerations include reusability of the lipase, choice of substrate to obtain maximum yields of cocoa butter-like triacylglycerols and the cost of extraction and purification.

Modification of hardness and melting properties

The physical properties of fats can be modified via chemical or enzymatic methods (Marangoni and Rousseau, 1995). The effects of blending and chemical interesterification on the physical and chemical properties of fats have been extensively studied (Rousseau *et al.*, 1996a,b,c; Marangoni and Rousseau, 1998a,b; Rousseau and Marangoni, 1998a,b), however, almost no information exists on the effects of enzymatic interesterification on the physical properties of fats. In order to be able to use such methods in the manufacture of new fat-containing products, it is imperative to understand the effects of enzymatic transformations on the physical properties of fats.

Rousseau and Marangoni (1998a,b) enzymatically interesterified milkfat using *Rhizopus arrhizus* lipase, which possesses long chain fatty acid and sn-1,3 specificity, in order to produce a softer, cold-spreadable butter. The hardness index, a macroscopic measure of the solid-like character of a material, decreased as a function of interesterification duration in an exponential fashion (*Figure 7*). This decrease in the solid-like character of the milkfat was also observed using more sophisticated rheological measurements, such as dynamic oscillatory controlled-stress rheometry. In these experiments, the shear storage modulus G' (or elastic component), as well as

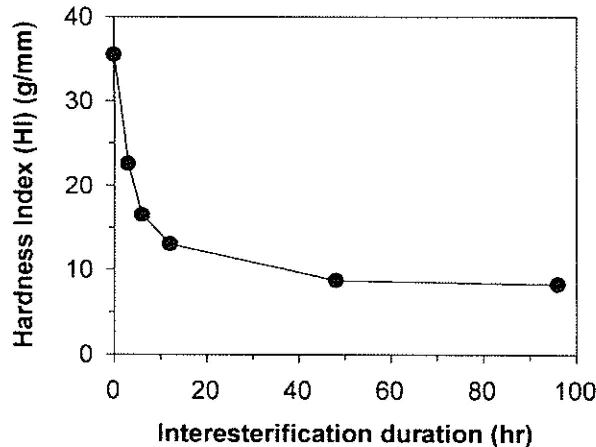

Figure 7. Hardness index as a function of time for interesterification of milkfat using lipase from *Rhizopus arrhizus*.

the shear loss modulus G" (or viscous component), decreased upon lipase-catalysed interesterification.

This decrease in the hardness of the fat was mainly attributed to a decrease in the solid-fat-content, as determined by pulsed NMR (*Figure 8*), and not to changes in the microstructure of the fat crystal network. The dropping point, a measure of the end of melt of the fat, also decreased upon enzymatic interesterification. This decrease in dropping point suggested alterations in the structure of the triglycerides, which in turn lead to changes in their ability to interact in the solid-state. This hypothesis was confirmed by X-ray diffraction studies on the milkfat where the size of the unit cell (long-spacings) increased from 4.20 nm to 4.54 nm upon enzymatic interesterification. This information, combined with the fact that the main subcell reflections (short-spacings) decreased from 0.400 and 0.433 nm to 0.392 and 0.429 nm upon enzymatic interesterification suggested that the triglycerides were crystallizing predominantly in the orthorhombic perpendicular, or β', polymorph. The packing density of TAGs in this polymorphic state is lower and the melting point is lower than for triclinic, or β, polymorph. Therefore, the decrease in the hardness of milkfat upon enzymatic interesterification was attributed mainly to a decrease in the amount of solid fat present and a slight increase in the amount of β'-crystallizing triglyceride species.The advantages of using lipases over chemical means to modify the physical properties of fats and oils are not completely clear. Further understanding of the impact of the positional distribution of different fatty acids on the physical properties of fats and oils is required.

Conclusions

The major barrier to applying biotechnology to lipid modification, in the form of genetic engineering, biotransformation and lipase-catalysed interesterification remains cost. Present chemical modification methods are relatively inexpensive, easy to run and easy to scale-up, with the major difference being a more limited ability to manufacture products of differing fatty acid composition.

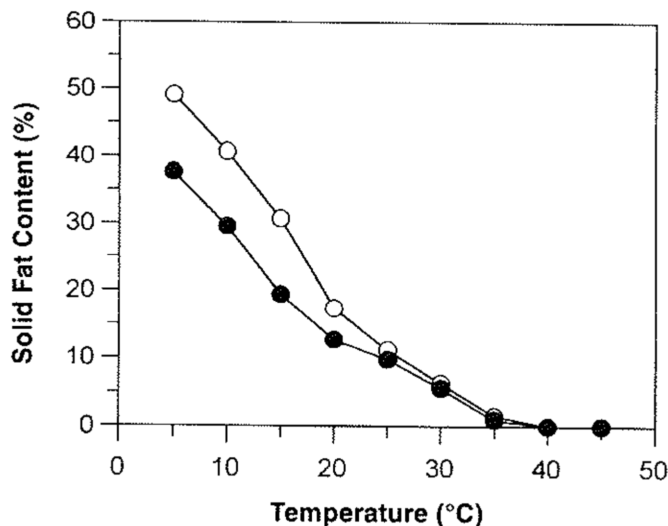

Figure 8. Solid fat content as a function of temperature for milkfat interesterified using lipase from *Rhizopus arrhizus*. (O—O) Milkfat, (●—●) interesterified milkfat.

Genetic engineering of oilseed crops and positional distribution has the greatest start-up expense but may prove to have the greatest number of applications in the long run. Structured lipids and production of PUFAs in oilseeds are definite possibilities as greater understanding of the processes involved is acquired.

Extensive studies have been performed using lipases, however, scale-up of lipase catalysed reactions is not economical at the moment in applications towards the nutritional modification of fats and oils. The high costs of lipases, reactor systems and process control are some of the main barriers. Biotransformations to produce n-3 PUFAs have been relatively successful so far in terms of larger scale production of specialty fats and oils for nutritional applications.

Overall, biotechnology is a valuable tool in the modification of fats and oils to meet the diverse needs of consumers, however, continued cost reduction and production optimization are required.

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References

ACKMAN, R. (1988). The year of the fish oils. *Chem. Ind.* **5**,139–144.

ADACHI, S., OKUMURA, K., OTA, V. AND MANKURA, M. (1993). Acidolysis of sardine oil by lipase to concentrate eicosapentaenoic and docosahexaenoic acids in glycerides. *J. Ferment. Bioeng.* **75**, 259–264.

AKIMOTO, M., ISHII, T., YAMAGAKI, K., OHTAGUCHI, K., KOIDE, K. AND YAZAWA, K. (1990).

Production of eicosapentaenoic acid by a bacterium isolated from mackerel intestines. *J. Am. Oil Chem. Soc.* **67**, 911–915.

AKIMOTO, M., ISHII, T., YAMAGAKI, K., OHTAGUCHI, K., KOIDE, K. AND YAZAWA, K. (1991). Metal salts requisite for the production of eicosapentaenoic acid by a marine bacterium isolated from mackerel intestines. *J. Am. Oil Chem. Soc.* **68**, 504–508.

AKOH, C.C., JENNINGS, B.H. AND LILLARD, D.A. (1995). Enzymatic modification of trilinolein: incorporation of n-3 polyunsaturated fatty acids. *J. Am. Oil Chem. Soc.* **72**, 1317–1321.

AKOH, C.C., JENNINGS, B.H. AND LILLARD, D.A. (1996). Enzymatic modification of evening primrose oil: incorporation of n-3 polyunsaturated fatty acids. *J. Am. Oil Chem. Soc.* **73**, 1059–1062.

BABAYAN, V.K. AND ROSENAU, J.R. (1991). Medium-chain triglycerides in cheese. *Food Technol.* **45**, 111–114.

BACH, A.C., STORCK, D. AND MERAIHI, Z. (1988). Medium-chain triglyceride-based fat emulsions: an alternative energy supply in stress and sepsis. *J. Parent. Enteral Nutr.* **12**, 82S–87S.

BAJPAI, P. AND BAJPAI, P.K. (1993). Eicosapentaenoic acid (EPA) production from microorganisms: a review. *J. Biotech.* **30**, 161–183.

BAJPAI, P., BAJAPI, P.K. AND WARD, O.P. (1991). Optimization of production of docosahexaenoic acid (DHA) by *Thraustochytrium aureum* ATCC 34304. *J. Am. Oil Chem. Soc.* **68**, 510–514.

BANG, H.O. AND DYERBERG, J. (1972). Plasma lipids and lipoproteins in Greenlandic West Coast Eskimos. *Acta. Med. Scand.* **192**, 85–94.

BECH PEDERSEN, S. AND HOLMER, C. (1995). Studies of the fatty acid specificity of the lipase from *Rhizomucor miehei* toward 20:1n-9, 20:5n-3, 22:1n-9 and 22:6n-3. *J. Am. Oil Chem. Soc.* **72**, 239–243.

BELL, S.J., BRADLEY, D., FORSE, R.A. AND BISTRIAN, B.R. (1997). The new dietary fats in health and disease. *J. Am. Diet. Assoc.* **97**, 280–286.

BERDANIER, C.D. (1995). Lipids. In *Advanced Nutrition: Macronutrients.* pp 220–222, 234–236. Boca Raton, Florida: CRC Press, Inc.

BLOOMER, S., ADLERCREUTZ, P. AND MATTIASSON, B. (1990). Triglyceride interesterification by lipases. 1. Cocoa butter equivalents from a fraction of palm oil. *J. Am. Oil Chem. Soc.* **67**, 519–524.

BOEHM, G., MÜLLER, H., KOHN, G., MORO, G., MINOLI, I. AND BÖHLES, H.J. (1997). Docosahexaenoic and arachidonic acid absorption in preterm infants fed LCP-free or LCP-supplemented formula in comparison to infants fed fortified breast milk. *Ann. Nutr. Metab.* **41**, 235–241.

BORUM, P.R. (1992). Medium-chain triglycerides in formula for preterm neonates: implications for hepatic and extrahepatic metabolism. *J. Pediatr.* **120**, S139–S145.

BRADEN, L.M. AND CARROLL, K.K. (1986). Dietary polyunsaturated fat in relation to mammary carcinogenesis in rats. *Lipids* **21**, 285–288.

BRUCKNER, G. (1992). Biological effects of polyunsaturated fatty acids. In *Fatty Acids in Foods and Their Health Implications.* Ed. C.K. Chow, pp 631–646. New York: Marcel Dekker, Inc.

BURGESS, J.G., IWAMOTO, K., MIURA, Y., TAKANO, H., MATSUNAGA, T. (1993). An optical fibre photobioreactor for enhanced production of the marine unicellular alga *Isochrysis* aff. *Galbana* T-Iso (UTEX LB 2307) rich in docosahexaenoic acid. *Appl. Microbiol. Biotechnol.* **39**, 456–459.

CAGGIULA, A.W. AND MUSTAD, V.A. (1997). Effects of dietary fat and fatty acids on coronary artery disease risk and lipoprotein cholesterol concentrations: epidemiologic studies. *Am. J. Clin. Nutr.* **65**, 1597S–1610S.

CARLSON, S.E. (1996). Arachidonic acid status of human infants: Influence of gestational age at birth and diets with very long chain n-3 and n-6 fatty acids. *J. Nutr.* **126**, 1092S–1098S.

CARLSON, S.E., CLANDININ, M.T., COOK, H.W., EMKEN, F.A. AND FILER, L.J. JR. (1997). *trans* Fatty acids: infant and fetal development. *Am. J. Clin. Nutr.* **66**, 715S–736S.

CARLSON, S.E., COOKE. R.J., WERKMAN, S.H. AND TOLLEY, E.A. (1992). First year growth of preterm infants fed standard compared to marine-oil n-3 supplemented formula. *Lipids* **27**, 901–907.

CARLSON, S.E., WERKMAN, S.H., RHODES, P.G. AND TOLLEY, E.A. (1993). Visual-acuity development in healthy preterm infants: effect of marine-oil supplementation. *Am. J. Clin. Nutr.* **58**, 35–42.

CARLSON, S.E. AND WERKMAN, S.H. (1996). A randomized trial of visual attention of preterm infants fed docosahexaenoic until two months. *Lipids* **31**, 85–89.

CARLSON, S.E., FORD, A.J., WERKMAN, S.H., PEEPLES, J.M. AND KOO, W.W.K. (1996). Visual acuity and fatty acid status of term infants fed human milk and formulas with and without docosahexaenoate and arachidonate from egg yolk lecithin. *Pediatr. Res.* **39**, 882–888.

CARNIELLI, V.P., LUIJENDIJK, I.H.T., VAN GOUDOEVER, J.B., SULKERS, E.J., BOERLAGE, A.A., DEGENHART, H.J. AND SAUER, P.J.J. (1995). Feeding premature newborn infants palmitic acid in amounts and stereochemical position similar to that of human milk: effects on fat and mineral balance. *Am. J. Clin. Nutr.* **61**, 1037–1042.

CARNIELLI, V.P., PEDERZINI, F., VITTORANGELI, R., LUIJENDIK, I.H.T, BOMAARS, W.E.M., PEDROTTI, D. AND SAUER, P.J.J. (1996). Plasma and red blood cell fatty acid of very low birth weight infants fed exclusively with expressed preterm human milk. *Pediatr. Res.* **39**, 671–679.

CARNIELLI, V.P., VERLATO, G., PEDERZINI, F., LUIJENDIJK, I., BOERLAGE, A., PEDROTTI, D. AND SAUER, P.J.J. (1998). Intestinal absorption of long-chain polyunsaturated fatty acids in preterm infants fed breast milk or formula. *Am. J. Clin. Nutr.* **67**, 97–103.

CARTENS, M., MOLINA GRIMA, E., ROBLES MEDINA, A., GIMÉNEZ GIMÉNEZ, A. AND IBÁÑEZ GONZÁLES, J. (1996). Eicosapentaenoic acid (20:5n-2) from the marine microalga *Phaeodactylum tricornutum*. *J. Am. Oil Chem. Soc.* **71**, 1025–1034.

CHANG, M.-K., ABRAHAM, G. AND JOHN, V.T. (1990). Production of cocoa butter-like fat from interesterification of vegetable oils. *J. Am. Oil Chem. Soc.* **67**, 832–834.

CHONG, C.N., HOH, Y.M. AND WANG, C.W. (1992). Fractionation procedures for obtaining cocoa-butter-like fat from enzymatically interesterified palm olein. *J. Am. Oil Chem. Soc.* **69**, 137–140.

CHRISTENSEN, M.S., HØY, C.-E., BECKER, C.C. AND T.G. REDGRAVE. (1995). Intestinal absorption and lymphatic transport of eicosapentaenoic (EPA), docosahexaenoic (DHA), and decanoic acids: dependence on intramolecular triacylglycerol structure. *Am. J. Clin. Nutr.* **61**, 56–61.

CHRISTENSEN, M.S. AND HØY, C-E. (1997). Early dietary intervention with structured triacylglycerols containing docosahexaenoic acid. Effect on brain, liver and adipose tissue lipids. *Lipids* **32**, 185–191.

COENEN, J.W.E. (1974). Fractionnement et interestérification des corps gras dans la perspective du marché mondial des matières premières et des produits finis, II – interestérification. *Rev. Franç. Corps Gras.* **21**, 403–413.

COHEN, Z. (1990). The production potential of eicosapentaenoic and arachidonic acids by the red alga *Porphyridum cruentum*. *J. Am. Oil Chem. Soc.* **67**, 916–919.

CRAWFORD, M.A., COSTELOE, K., GHEBREMESKEL, K., PHYLACTOS, A., SKIRVIN, L. AND STACEY, F. (1997). Are deficits of arachidonic and docosahexaenoic acids responsible for the neural and vascular complications of preterm babies? *Am. J. Clin. Nutr.* **66 (suppl)**, 1032S–1041S.

DAVIES, H.M. (1993). Medium chain acyl-ACP hydrolysis activities of developing oilseeds. *Phytochem.* **33**, 1353–1356.

DE FOUW, N.J., KIVITS, G.A.A., QUINLAN, P.T. AND VAN NIELEN, W.G.L. (1994). Absorption of isomeric, palmitic acid-containing triacylglycerols resembling human milk fat in the adult rat. *Lipids* **29**, 765–770.

DEHESH, K., JONES, A. KNUTZON, D.S., VOELKER, T.A. (1996). Production of high levels of 8:0 and 10:0 fatty acids in transgenic canola by overexpression of *Ch FatB2*, a thioesterase cDNA from *Cuphea hookeriana*. *Plant J.* **9**, 167–172.

DEL VECCHIO, A.J. (1996). High-laurate canola. *Inform.* **7**, 230–243.

DENKE, M.A. AND GRUNDY, S.M. (1992). Comparison of effects of lauric acid and palmitic acid on plasma lipids and lipoproteins. *Am. J. Clin. Nutr.* **56**, 895–898.

DESCI, T. AND KOLETZKO, B. (1995). Do trans fatty acids impair linoleic acid metabolism in children? *Ann. Nutr. Metab.* **39**, 36–41.

DESNUELLE, P. (1972). The Lipases, In *The Enzymes*, Volume VII, 3rd edition. Ed. P.D. Boyer, pp 575–616. New York: Academic Press.

FAN, S.T. (1997). Review: nutritional support for patients with cirrhosis. *J. Gastroenterol. Hepatol.* **12**, 282–286.

FERRARI, R. AP., ESTEVES, W. AND MUKHERJEE, K.D. (1997). Alteration of steryl ester content and positional distribution of fatty acids in triacylglycerols by chemical and enzymatic interesterification of plant oils. *J. Am. Oil Chem. Soc.* **74**, 93–96.

FITCH-HAUMANN, B. (1997). Bioengineered oilseed acreage escalating. *Inform.* **8**, 804–811.

FRANCOIS, C.A., CONNOR, S.L., WANDER, R.C. AND CONNOR, W.E. (1998). Acute effects of dietary fatty acids on the fatty acids of human milk. *Am. J. Clin. Nutr.* **67**, 301–308.

FREDRIKZON, B. AND OLIVECRONA, T. (1978). Decrease of lipase and esterase activities in intestinal contents of newborn infants during test meals. *Pediatr. Res.* **12**, 631–634.

FUKUDA, H. AND MORIKAWA, H. (1987). Enhancement of γ-linolenic acid production by *Mucor ambiguus* with non-ionic surfactants. *Appl. Microbiol. Biotechnol.* **27**, 15–20.

GARG, M.L., THOMSON, A.B.R. AND CLANDININ, M.T. (1990). Interactions of saturated, n-6 and n-3 polyunsaturated fatty acids to modulate arachidonic acid metabolism. *J. Lipid Res.* **31**, 271–277.

GHAZALI, H.M., HAMIDAH, S. AND CHE MAN, Y.B. (1995). Enzymatic transesterification of palm olein with nonspecific and 1,3-specific lipases. *J. Am. Oil Chem. Soc.* **72**, 633–639.

GHOSH, S. AND BHATTACHARYYA, D.K. (1997). Medium-chain fatty acid-rich glycerides by chemical and lipase-catalyzed polyester-monoester interchange reaction. *J. Am. Oil. Chem. Soc.* **74**, 593–595.

GIBSON, R.A., MAKRIDES, M., NEUMANN, M.A., SIMMER, K., MANTZIORIS, E. AND JAMES, M.J. (1994). Ratios of linoleic acid to alpha-linolenic acid in formulas for term infants. *J. Pediatr.* **125**, S48–S55.

GRUNDY, S.M. (1997). What is the desirable ratio of saturated, polyunsaturated, and monounsaturated fatty acids in the diet? *Am. J. Clin. Nutr.* **66 (suppl)**, 988S–990S.

GUNSTONE, F.D. (1994). Marine oils: fish and whale oils. In *The Lipid Handbook*, 2nd edition. Eds. F.D. Gunstone, J.L. Harwood, F.D. Padley, p 177. London: Chapman and Hall.

GURR, M.I. (1992). *The Role of Fats in Food and Nutrition*, pp 123, 131–133, V.

HANSSON, L. AND DOSTÁLEK, M. (1988). Effect of culture conditions on mycelial growth and production of γ-linolenic acid by the fungus *Mortiella ramanniana*. *Appl. Microbiol. Biotechnol.* **28**, 240–246.

HARDY, S.C. AND KLEINMAN, R.E. (1994). Fat and cholesterol in the diet of infants and young children: implications for growth, development and long-term health. *J. Pediatr.* **125**, S69–S77.

HARALDSSON, G.G., GUDMUNSSON, B.Ö. AND ALMARSSON, Ö. (1993). The preparation of homogeneous triglycerides of eicosapentaenoic acid and docosahexaenoic acid by lipase. *Tetrahedron Lett.* **34**, 5791–5794.

HILLS, M.J., KLEWITT, I. AND MUKHERJEE, K.D. (1990). Enzymatic fractionation of fatty acids: enrichment of gamma-linolenic acid and docosahexaenoic acid by selective esterification catalyzed by lipases. *J. Am. Oil Chem. Soc.* **67**, 561–564.

HOSHINO, T., YAMANE, T. AND SHIMIZU, S. (1990). Selective hydrolysis of fish oil by lipase to concentrate n-3 polyunsaturated fatty acids. *Agric. Biol. Chem.* **54**, 1459–1467.

HUANG, K. AND AKOH, C.C. (1996). Enzymatic synthesis of structured lipids: Transesterification of triolein and caprylic acid ethyl ester. *J. Am. Oil Chem. Soc.* **73**, 245–250.

HUANG, K. AND AKOH, C.C. (1994). Lipase-catalyzed incorporation of n-3 polyunsaturated fatty acids into vegetable oils. *J. Am. Oil Chem. Soc.* **71**, 1277–1280.

HUANG, Y.-S., LIN, X., REDDEN, P.R. AND HORROBIN, D.F. (1995). *In vitro* hydrolysis of natural and synthetic γ-linolenic acid-containing triacylglycerols by pancreatic lipase. *J. Am. Oil Chem. Soc.* **72**, 625–631.

IIDA, I., NAKAHARA, T., YOKOCHI, T., KAMISAKA, Y., YAGI, H., YAMAOKA, M. AND SUZUKI, O. (1996). Improvement of docosahexaenoic acid production in a culture of *Thraustochytrium aureum* by medium optimization. *J. Ferment. Bioeng.* **81**, 76–78.

INNIS, S.M. (1992). n-3 Fatty acid requirements of the newborn. *Lipids* **27**, 879–885.

INNIS, S.M., AKRABAWI, S.S., DIERSEN-SCHADE, D.A., DOBSON, M.V. AND GUY, D.G. (1997). Visual acuity and blood lipids in term infants fed human milk or formulae. *Lipids* **32**, 63–72.

INNIS, S.M., DYER, R., QUINLAN, P. AND DIERSEN-SCHADE, D. (1995). Palmitic acid is absorbed as sn-2 monopalmitin from milk and formula with rearranged triacylglycerols and results in increased plasma triglyceride sn-2 and cholesterol ester palmitate in piglets. *J. Nutr.* **125**, 73–81.

INNIS, S.M., NELSON, C.M., LWANGA, D., RIOUX, F.M. AND WASLEN, P. (1996). Feeding formula without arachidonic acid and docosahexaenoic acid has no effect on preferential looking acuity or recognition memory in healthy full-term infants at 9 mo of age. *J. Clin. Nutr.* **64**, 40–46.

INNIS, S.M., NELSON, C.M., RIOUX, M.F. AND KING, D.J. (1994). Development of visual acuity in relation to plasma and erythrocyte w-6 and w-3 fatty acids in healthy term gestation infants. *Am. J. Clin. Nutr.* **60**, 347–352.

JAEGER, K-E., RANSAC, S., DIJKSTRA, B.W., COLSON, C.,VAN HEUVEL, M. AND MISSET, M. (1995). Bacterial lipases. *FEMS Microbiol. Rev.* **15**, 29–63.

JEEVANANDAM, M., HOLADAY, N.J., VOSS, T., BUIER, R. AND PEDERSEN, S.R. (1995). Efficacy of a mixture of medium-chain triglyceride (75%) and long-chain triglyceride (25%) fat emulsions in the nutritional management of multiple-trauma patients. *Nutrition* **11**, 275–284.

JOHNSON, R.C., YOUNG, S.K., COTTER, R., LIN, L. AND ROWE, W.B. (1990). Medium-chain-triglyceride lipid emulsion: metabolism and tissue distribution. *Am. J. Clin. Nutr.* **52**, 502–508.

JOOSSENS, J.V., GEBOERS, J. AND KESTELOOT, H. (1989). Nutrition and cardiovascular mortality in Belgium. *Acta Cardiol.* **44**, 157–182.

KAMISAKA, Y., TOSHIHIRO, Y., NAKAHARA, T. AND SUKZUKI, O. (1990). Incorporation of Linoleic acid and its conversion to γ-linolenic acid in fungi. *Lipids* **25**, 54–60.

KAWACHI, M., KATO, M., IKEMOTO, H. AND MIYACHI, S. (1996). Fatty acid composition of a new marine picoplankton species of the Chromophyta. *J. Appl. Phycol.* **8**, 397–401.

KENDRICK, A. AND RATLEDGE, C. (1992). Lipids of selected molds grown for production of n-3 and n-6 polyunsaturated fatty acids. *Lipids* **27**, 15–20.

KLEMANN, L.P., AJI, K., CHRYSAM, M.M., D'AMELIA, R.P., HENDERSON, J.M., HUANG, A.S., OTTERBURN, M.S. AND YARGER, R.G. (1994). Random nature of triacylglycerols produced by the catalyzed interesterification of short and long-chain fatty acid triglycerides. *J. Agric. Food Chem.* **42**, 442–446.

KNAPP, S.J., TAGLIANI, L.A. AND ROATH, W.W. (1991). Fatty acid and oil diversity of *Cuphea viscosissima*: a source of medium-chain fatty acids. *J. Am. Oil Chem. Soc.* **68**, 515–517.

KNUTZON, D.S., LARDIZABAL, K.D., NELSEN, J.S., BLEIBAUM, J.L., DAVIES, H.M. AND METZ, J.G. (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.

KOON TAN, C. AND JOHNS, M.R. (1996). Screening of diatoms for heterotrophic eicosapentaenoic acid production. *J. Appl. Phycol.* **8**, 59–64.

KOSUGI, Y. AND AZUMA, N. (1994). Synthesis of triacylglycerol from polyunsaturated fatty acid by immobilized lipase. *J. Am. Oil Chem. Soc.* **71**, 1397–1403.

KUKSIS, A., MCCARTHY, M.J. AND BEVERIDGE, J.M.R. (1963). Triglyceride composition of native and rearranged butter and coconut oils. *J. Am. Oil Chem. Soc.* **41**, 201–205.

KRIS-ETHERTON, P.M. AND YU, S. (1997). Individual fatty acid effects on plasma lipids and lipoproteins: human studies. *Am. J. Clin. Nutr.* **65**, 1628S–1644S.

KROKAN, H.E., BJERVE, K.S. AND MØRK, E. (1983). The enteral bioavailability of eicosapentaenoic acid and docosahexaenoic acid is as good from ethyl esters as from glyceryl esters in spite of lower hydrolytic rates by pancreatic lipase *in vitro*. *Biochim. Biophys. Acta* **1168**, 59–67.

KWON, D.Y., SONG, H.N. AND YOON, S.H. (1996). Synthesis of medium-chain glycerides by lipase in organic solvent. *J. Am. Oil Chem. Soc.* **73**, 1521–1525.

LAMBERT, M.S., BOTHAM, K.M. AND MAYES, P.A. (1996). Modification of the fatty acid

composition of dietary oils and fats on incorporation into chylomicrons and chylomicron remnants. *British J. Nutr.* **76**, 435–445.

LANGHOLZ, P., ANDERSEN, P., FORSKOV, T. AND SCHMIDTSDORFF, W. (1989). Application of specificity of *Mucor miehei* lipase to concentrate docosahexaenoic acid (DHA). *J. Am. Oil Chem. Soc.* **66**, 1120–1123.

LEE, K-T. AND AKOH, C.C. (1996). Immobilized lipase-catalyzed production of structured lipids with eicosapentaenoic acid at specific positions. *J. Am. Oil Chem. Soc.* **73**, 611–615.

LEE, K-T. AND AKOH, C.C. (1997). Effects of selected substrate forms on the synthesis of structured lipids by two immobilized lipases. *J. Am. Oil Chem. Soc.* **74**, 579–584.

LI, Z-Y. AND WARD, O.P. (1993a). Enzyme-catalysed production of vegetable oils containing omega-3 polyunsaturated fatty acid. *Biotechnology Lett.* **15**, 185–188.

LI, Z-Y. AND WARD, O.P. (1993b). Lipase-catalyzed esterification of glycerol and n-3 polyunsaturated fatty acid concentrate in organic solvent. *J. Am. Oil Chem. Soc.* **70**, 745–748.

LIE KEN JIE, M.S.F. AND SYED RAHMATULLAH, M.S.K. (1995). Enzymatic enrichment of C_{20} *cis*-5 polyunsaturated fatty acids from *Biota orientalis* seed oil. *J. Am. Oil Chem. Soc.* **72**, 245–249.

LINDBERG, A.-M. AND MOLIN, G. (1993). Effect of temperature and glucose supply on the production of polyunsaturated fatty acids by the fungus *Mortiella alpina* CBS 343.66 in fermentor cultures. *Appl. Microbiol. Biotechnol.* **39**, 450–455.

LINKO, Y-Y. AND HAYAKAWA, K. (1996). Docosahexaenoic acid: a valuable nutraceutical? *Trends Food Sci. Technol.* **7**, 59–63.

LÓPEZ ALONSO, D. AND SEGURA DEL CASTILLO, C.I. (1996). First insights into improvement of eicosapentaenoic acid content in *Phaeodactylum tricornutum* (bacillariophyceae) by induced mutagenesis. *J. Phycol.* **32**, 339–345.

MACRAE, A.R. (1983). Lipase-catalyzed interesterification of oils and fats. *J. Am. Oil Chem. Soc.* **60**, 291–294.

MACRAE, A.R. (1985). Interesterification of fats and oils. In *Biocatalysts in Organic Syntheses*. Eds. J. Tramper, H. C. van der Plas and P. Linko, Proceedings of an International Symposium held at Noorwijkerhout, The Netherlands (14–17 April 1985). pp 195–208. Amsterdam: Elsevier Science Publishers.

MACRAE, A.R. AND HOW, P. (1988). Rearrangement Process, *United States Patent*, patent number **4**, 719, 178.

MAKRIDES, M., NEUMANN, M.A. AND GIBSON, R.A. (1996). Is dietary docosahexaenoic acid essential for term infants. *Lipids* **31**, 115–119.

MAKRIDES, M., NEUMANN, M.A., SIMMER, K. AND GIBSON, R.A. (1995). Erythrocyte fatty acids of term infants fed either breast milk, standard formula, or formula supplemented with long-chain polyunsaturates. *Lipids* **30**, 941–948.

MALCATA, F.X., REYES, H.R., GARCIA, H.S., HILL, C.G. AND AMUNDSON, C.H. (1992). Kinetics and mechanisms catalyzed by immobilized lipases. *Enzyme Microb. Technol.* **14**, 426–446.

MARANGONI, A.G. AND ROUSSEAU, D. (1995). Engineering triacylglycerols: the role of interesterification. *Trends Food Sci. Technol.* **6**, 329–335.

MARANGONI, A.G. AND ROUSSEAU, D. (1998a). The influence of chemical interesterification on the physiochemical properties of complex fat systems. I. Melting and crystallization. *J. Am. Oil Chem. Soc.* **75**, 1265–1271.

MARANGONI, A.G. AND ROUSSEAU, D. (1998b). The influence of chemical interesterification on the physiochemical properties of complex fat systems. III. Rheology and fractality of the fat crystal network. *J. Am. Oil Chem. Soc.* **75**, 1633–1636.

MARTINEZ, M. (1992). Tissue levels of polyunaturated fatty acids during early human development. *J. Pediatr.* **120 (suppl)**, S129–S138.

MASCIOLI, E.A., BABAYAN, V.K., BISTRIAN, B.R. AND BLACKBURN, G.L. (1988). Novel triglycerides for special medical purposes. *J. Parent. Enteral Nutr.* **12**, 127S–131S.

MCGEE, D.L., REED, D.M., YANO, K., KAGAN, A. AND TILLOTSON, J. (1984). Ten year incidence of coronary heart disease in Honolulu Heart Program. Relationship to nutrient intake. *Am. J. Epidemiol.* **119**, 667–676.

MCNEILL, G.P., ACKMAN, R.G. AND MOORE, S.R. (1996). Lipase-catalyzed enrichment of long-chain polyunsaturated fatty acids. *J. Am. Oil Chem. Soc.* **73**, 1403–1407.

MEDINA, A.R., GIMÉNEZ GIMÉNEZ, A., GARCÍA CAMACHO, F., SÁNCHEZ PÉREZ, J.A., MOLINA GRIMA, E. AND CONTRERAS GÓMEZ, A. (1995). Concentration and purification of stearidonic, eicosapentaenoic and docosahexaenoic acids from cod liver oil and the marine microalgae *Isochyrysis galbana*. *J. Am. Oil Chem. Soc.* **72**, 575–581.

MEGREMIS, C.J. (1991). Medium-chain triglycerides: a nonconventional fat. *Food Tech.* **45**, 108–110.

MENSINK, R.P. AND KATAN, M.B. (1990). Effect of dietary trans fatty acids on high-density and low-density lipoprotein cholesterol levels in healthy subjects. *New Eng. J. Med.* **323**, 439–444.

MIQUEL, M. AND BROWSE, J. (1995). Molecular biology of oilseed modification. *Inform.* **6**, 108–111.

MOLINA GRIMA, E., ROBLES MEDINA, A., GIMÉNEZ GIMÉNEZ, A. AND IBÁÑEZ GONZÁLES, M.J. (1996). Gram-scale purification of eicosapentaenoic acid (EPA, 20:5n-3) from wet *Phaeodactylum tricornutum* UTEX 640 biomass. *J. Appl. Phycol.* **8**, 359–367.

MOLINA GRIMA, E., SÁNCHEZ PÉREZ, J.A., GARCÍA CAMACHO, F., GARCÍA SÁNCHEZ, J.L. AND FERNÁNDEZ SEVILLA, J.M. (1995). Variation of fatty acid profile with solar cycle in outdoor chemostat culture of *Isochrysis galbana* ALII-4. *J. Appl. Phycol.* **7**, 129–134.

MOJOVIC, L., ŠILER-MARINKOVIC, S., KUKIC, G. AND VUNJAK-NOVAKOVIC. (1993). *Rhizopus arrhizus* lipase-catalyzed interesterification of the midfraction of palm oil to a cocoa butter equivalent fat. *Enzyme Microb. Technol.* **15**, 438–443.

MUKHERJEE, K.D. (1998). Lipid Biotechnology. In *Food Lipids: Chemistry, Nutrition and Biotechnology*. Eds. C.C. Akoh and D.B. Min, pp 589–640. New York: Marcel Dekker, Inc.

MURPHY, D.J. (1994). Manipulation of lipid metabolism in transgenic plants: biotechnological goals and biochemical realities. *Biochemical Soc. Trans.* **22**, 926–931.

MURPHY, D.J. (1996). Engineering oil production in rapeseed and other oil crops. *Tibtech.* **14**, 206–213.

MYRNES, B., BARSTAD, H., OLSEN, R.L. AND ELVEVOLL, E.O. (1995). Solvent-free enzymatic glycerolysis of marine oils. *J. Am. Oil Chem. Soc.* **72**, 1339–1344.

NAKAHARA, T., YOKOCHI, T., HIGASHIHARA, T., TANAKA, S., YAGUCHI, T. AND HONDA, D. (1996). Production of docosahexaenoic and docosapentaenoic acids by *Schizochytrium* sp. isolated from Yap Islands. *J. Am. Oil Chem. Soc.* **73**, 1421–1426.

NAWAR, W.W. (1996). Chemistry. In *Bailey's Industrial Oil and Fat Products, Volume 1: Edible Oil and Fat Products, General Applications*, 5th Edition. Ed. Y.H. Hui, pp 232, 409. New York: John Wiley and Sons, Inc.

NELSON, G.J. (1992). Dietary fatty acids and lipid metabolism, In *Fatty Acids in Foods and Their Health Implications*. Ed. C.K. Chow, pp 437–365. New York: Marcel Dekker.

NELSON, G.J. AND ACKMAN, R.G. (1988). Absorption and transport of fat in mammals with emphasis on n-3 polyunsaturated fatty acids. *Lipids* **23**, 1005–1014.

NEURINGER, M., REISBICK, S. AND JANOWSKY, J. (1994). The role of n-3 fatty acids in visual and cognitive development: current evidence and methods of assessment. *J. Pediatr.* **125**, S39–S47.

NORDENSTRÖM, J., THÖRNE, A. AND OLIVECRONA, T. (1995). Metabolic effects of infusion of a structured-triglyceride emulsion in healthy individuals. *Nutrition* **11**, 269–274.

O'BRIEN, D., KURANTZ, M.J. AND KWOCZAK, R. (1993). Production of eicosapentaenoic acid by the filamentous fungus *Pythium irregulare*. *Appl. Microbiol. Biotechnol.* **40**, 211–214.

OHLROGGE, J.B. (1983). Distribution in human tissues of fatty acid isomers from hydrogenated oils. In *Dietary Fats and Health*. Eds. E.G. Perkins, E.G. and W.J. Visek, p 359. Champaign, Illinois: American Oil Chemists' Society.

OHLROGGE, J.B. (1994). Design of new plant products: engineering of fatty acid metabolism. *Plant. Physiol.* **104**, 821–826.

OSADA, K., TAKAHASHI, K. AND HATANO, M. (1990). Polyunsaturated fatty glyceride syntheses by microbial lipases. *J. Am. Oil Chem. Soc.* **67**, 921–922.

PAI, T. AND YEH, Y.-Y. (1997). Stearic acid modifies very low density lipoprotein lipid

composition and particle size differently from shorter chain saturated fatty acids in cultured rat hepatocytes. *Lipids* **32**, 143–149.

RATNAYAKE, W.M. AND CHEN, Z.Y. (1996). Trans, n-3 and n-6 fatty acids in Canadian human milk. *Lipids* **31**, S279–S282.

RAY, S. AND BHATTACHARYYA, D.K. (1995). Comparative nutritional study of enzymatically and chemically interesterified palm oil products. *J. Am. Oil Chem. Soc.* **72**, 327–330.

REDDY, T. AND THOMAS, T.L. (1996). Expression of a cyanobacterial D6-desaturase gene results in gamma-linolenic acid production in transgenic plants. *Nat. Biotechnol.* **14**, 639–642.

REYES, H.R. AND HILL, JR., C.G. (1994). Kinetic modeling of interesterification reactions catalyzed by immobilized lipase. *Biotechnol. Bioeng.* **43**, 171–182.

RIAUBLANC, R., RATOMAHENINA, R., GALZY, P. AND NICOLAS, M. (1993). Peculiar properties from *Candida parapsilosis* (Ashford) Langeron Talice. *J. Am. Oil Chem. Soc.* **70**, 497–500.

ROBLES-MEDINA, A., GIMÉNEZ GIMÉNEZ, A., GARCÍA CAMACHO, F., SÁNCHEZ PÉREZ, J.A., MOLINA GRIMA, E. AND CONTRERAS GÓMEZ, A. (1995). Concentration and purification of stearidonic, eicosapentaenoic and docosahexaenoic acids from cod liver oil and the marine microalga *Isochrysis galbana*. *J. Am. Oil Chem. Soc.* **72**, 575–583.

ROSE, A.H. (1989). Influence of the environment on microbial lipid composition. In *Microbial Lipids: Volume 2*. Eds. C. Ratledge and S.G. Wilkinson, pp 255–278. San Diego, USA: Academic Press.

ROUSSEAU, D. AND MARANGONI, A.G. (1998a). Tailoring the textural attributes of butterfat-canola oil blends via *Rhizopus arrhizus* lipase-catalyzed interesterification. I. Compositional modifications. *J. Agric. Food Chem.* **46**, 2368–2374.

ROUSSEAU, D. AND MARANGONI, A.G. (1998b). Tailoring the textural attributes of butterfat-canola oil blends via *Rhizopus arrhizus* lipase-catalyzed interesterification. 2. Modification of physical properties. *J. Agric. Food Chem.* **46**, 2375–2381.

ROUSSEAU, D., FORESTIERE, K., HILL, A.R. AND MARANGONI, A.G. (1996a). Restructuring butterfat through blending and chemical interesterification. I. Melting behaviour and triacylglyerol modifications. *J. Am. Oil Chem. Soc.* **73**, 963–972.

ROUSSEAU, D., HILL, A.R. AND MARANGONI, A.G. (1996b). Restructuring butterfat through blending and chemical interesterification. II. Microstructure and polymorphism. *J. Am. Oil Chem. Soc.* **73**, 973–981.

ROUSSEAU, D., HILL, A.R. AND MARANGONI, A.G. (1996c). Restructuring butterfat through blending and chemical interesterification. III. Rheology. *J. Am. Oil Chem. Soc.* **73**, 983–989.

SANDSTRÖM, R., HYLTANDER, A., KÖRNER, U. AND LUNDHOLM, K. (1993). Structured triglycerides to postoperative patients: a safety and tolerance study. *J. Parent. Enteral Nutr.* **17**, 153–157.

SHAPIRO, S. (1997). Do *trans* fatty acids increase the risk of coronary artery disease? A critique of the epidemiological evidence. *Am. J. Clin. Nutr.* **66(suppl)**, 1011S–1017S.

SHEKELLE, R.B., SHRYOCK, A.M. AND PAUL, O. (1981). Diet, serum cholesterol and death from coronary heart disease. The Western electric Study. *New Engl. J. Med.* **304**, 65–70.

SHIMADA, Y., MARUYAMA, K., OKAZAKI, S., NAKAMURA, M., SUGIHARA, A. AND TOMINAGA, Y. (1994). Enrichment of polyunsaturated fatty acids with *Geotrichum candidum* lipase. *J. Am. Oil Chem. Soc.* **71**, 951–954.

SHIMADA, Y., SUGIHARA, A., NAKANO, H., KURAMOTO, T., NAGAO, T., GEMBA, M. AND TOMINAGA, Y. (1997). Purification of docosahexaenoic acid by selective esterification of fatty acids from tuna oil with *Rhizopus delemar* lipase. *J. Am. Oil Chem. Soc.* **74**, 97–101.

SHIMADA, Y., SUGIHARA, A., NAKANO, H., YOKOTA, T., NAGAO, T., KOMEMUSHI, S. AND TOMINAGA, Y. (1996). Production of structured lipids containing essential fatty acids by immobilized *Rhizopus delemar* lipase. *J. Am. Oil Chem. Soc.* **73**, 1415–1420.

SHIMIZU, S., KAWASHIMA, H., AKIMOTO, K., SHINMEN, Y. AND YAMADA, H. (1989). Microbial conversion of an oil containing alpha-linolenic acid to an oil containing eicosapentaenoic acid. *J. Am. Oil Chem. Soc.* **66**, 343–347.

SHINMEN, Y., SHIMIZU, S., AKIMOTO, K., KAWASHIMA, H. AND YAMADA, H. (1989). Production of arachidonic acid by *Mortiella* fungi. *Appl. Microbiol. Biotechnol.* **31**, 11–16.

SHISHIKURA, A., FUJIMOTO, K. SUZUKI, T. AND ARAI, K. (1994). Improved lipase-catalyzed incorporation of long-chain fatty acids into medium chain triacylglycerides assisted by supercritical carbon dioxide extraction. *J. Am. Oil Chem. Soc.* **71**, 961–967.

SLABAS, A.R., SINDEN, B.S., CHASE, D., BROWN, A.P., COLEMAN, J., MARTINEZ-RIVAS, J. AND FAWCETT, T. (1992). In *Biochemistry and Molecular Biology of Membrane and Storage Lipids of Plants*. Eds. N. Murata and C. Somerville, Proceedings US/Japan Binational Seminar, Dec 13–17, 1992, Hawaii. Current Topics in Plant Physiology: An American Society of Plant Physiologists Series, Vol. 9. USA, pp 113–120.

SMALL, D.M. (1991). The effects of glyceride structure on absorption and metabolism. *Annu. Rev. Nutr.* **11**, 413–434.

SOBRADO, J., MOLDAWER, L.L., POMPOSELLI, J.J., MASCIOLI, E.A., BABAYAN, V.K., BISTRIAN, B.R. AND BLACKBURN, G.L. (1985). Lipid emulsions and reticuloendothelial system function in healthy and burned guinea pigs. *Am. J. Clin. Nutr.* **42**, 855–863.

SOUMANOU, M.M., BORNSCHEUER, U.T., MENGE, U. AND SCHMID, R.D. (1997). Synthesis of structured triglycerides from peanut oil with immobilized lipase. *J. Am. Oil Chem. Soc.* **74**, 427–433.

SPIELMANN, D., BRACCO, U., TRAITLER, H., CROZIER, G., HOLMAN, R., WARD, M. AND COTTER, R. (1988). Alternative lipids to usual w6 PUFAS: gamma-linolenic acid, alpha-linolenic acid, stearidonic acid, EPA, etc. *J. Parent. Enteral Nutr.* **12**, 111S–123S.

SRIDHAR, R. AND LAKSHMINARAYANA, G. (1992). Incorporation of eicosapentaenoic and docosahexaenoic acids into groundnut oil by lipase-catalyzed ester interchange. *J. Am. Oil Chem. Soc.* **69**, 1041–1042.

SRIDHAR, R., LAKSHMINARAYANA, G. AND KAIMAL, T.N.B. (1991). Modification of selected Indian vegetable fats into cocoa butter substitutes by lipase-catalyzed ester interchange. *J. Am. Oil Chem. Soc.* **68**, 726–730.

STAMATIS, H., XENAKIS, A., PROVELEGIOU, M. AND KOLISIS, F.N. (1993). Esterification reactions catalyzed by lipases in microemulsions: the role of enzyme location in relation to its selectivity. *Biotechnol. Bioeng.* **42**, 103–110.

TANAKA, Y., HIRANO, J. AND FUNADA, T. (1992). Concentration of docosahexaenoic acid in glyceride by hydrolysis of fish oil with *Candida cylindracea* lipase. *J. Am. Oil Chem. Soc.* **69**, 1210–1214.

TANAKA, Y., HIRANO, J. AND FUNADA, T. (1994). Synthesis of docosahexaenoic acid-rich triglyceride with immobilized *Chromobacterium viscosum* lipase. *J. Am. Oil Chem. Soc.* **71**, 331–334.

TOMARELLI, R.M. (1988). Suitable fat formulations for infant feeding. In *Dietary Fat Requirements in Health and Development*. Ed. J. Beare-Rogers, pp 1–28. USA: American Oil Chemists' Society, Library of Congress Cataloging-in-Publication Data.

TSO, P. AND WEDIMAN, S.W. (1987). Absorption and metabolism of lipid in humans. In *Lipids in Modern Nutrition*. Eds. M. Horisberger and U. Bracco, Nestlé Nutrition Workshop Series, Volume 13, pp 1–15. New York: Raven Press.

ULRICH, H., MCCARTHY PASTORES, S., KATZ, D.P. AND KVETAN, V. (1996). Parenteral use of medium-chain triglycerides: a reappraisal. *Nutrition* **12**, 231–238.

VAN ZYL, C.G., LAMBERT, E.V., HAWLEY, J.A., NOAKES, T.D. AND DENNIS, S.C. (1996). Effects of medium-chain triglyceride ingestion on fuel metabolism and cycling performance. *J. Appl. Physiol.* **80**, 2217–2225.

VERGROESEN, A.J. (1976). Early signs of polyunsaturated fatty acid deficiency. *Biblthca. Nutr. Dieta.* **23**, 19–25.

VOELKER, T.A., WORRELL, A.C., ANDERSEN, L., BLIEBAUM, L., FAN, C., HAWKINS, D., RADKE, S.E. AND DAVIES, H.M. (1992). Fatty acid biosynthesis redirected to medium chains in transgenic oilseed plants. *Science*. **257**, 72–73.

WATKINS, J.B. (1988). Lipid digestion in the developing infant. In *Dietary Fat Requirements in Health and Development*. Ed. J. Beare-Rogers, pp 29–42. USA: American Oil Chemists Society, Library of Congress.

WERKMAN, S.H. AND CARLSON, S.E. (1996). A randomized trial for visual attention of preterm infants fed docosahexaenoic acid until nine months. *Lipids* **31**, 91–97.

WESSINGER, E.W., O'BRIEN, D. AND KURANTZ, M.J. (1990). Identification of fungi for sweet

whey permeate utilization and eicosapentaenoic acid production. *J. Ind. Microbiol.* **6**, 191–197.

WIBERG, E. AND BAFOR, M. (1995). Medium chain-length fatty acids in lipids of developing oil palm kernel endosperm. *Phytochem.* **39**, 1325–1327.

WOLTER, F.P. (1993). Altering plant lipids by genetic engineering. *Inform.* **4**, 93–98.

YADAV, N., WIERZBICKI, A., KNOWLTON, S., PIERCE, J., RIPP, K., HITZ, W., AEGERTER, M. AND BROWSE, J. (1992). Genetic manipulation to alter fatty acid profiles of oilseed crops. In *Biochemistry and Molecular Biology of Membrane and Storage Lipids of Plants*. Eds. N. Murata and C. Somerville, Proceedings US/Japan Binational Seminar, Dec 13–17, 1992, Hawaii. Current Topics in Plant Physiology: An American Society of Plant Physiologists Series, Vol. 9. U.S.A., pp 60–66.

YADWAD, V.B., WARD, O.P. AND NORONHA, L.C. (1991). Application of lipase to concentrate the docosahexaenoic acid (DHA) fraction of fish oil. *Biotech. Bioeng.* **38**, 956–959.

YAMANE, T., SUZUKI, T. AND HOSHINO, T. (1993). Increasing n-3 polyunsaturated fatty acid content of fish oil by temperature control of lipase-catalyzed acidolysis. *J. Am. Oil Chem. Soc.* **70**, 1285–1287.

YANO, Y., NAKAYAMA, A., SAITO, H. AND ISHIHARA, K. (1994). Production of docosahexaenoic acid by marine bacteria isolated from deep sea fish. *Lipids* **29**, 527–528.

YONGMANITCHAI, W. AND WARD, O.P. (1991). Screening of algae for potential alternative sources of eicosapentaenoic acid. *Phytochemistry* **30**, 2963–2967.

YUAN, L. AND KNAUF, V.C. (1998). Modification of plant components. *Current Opin. Biotechnol.* **8**, 227–233.

ZOCK, P.L. AND KATAN, M.B. (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.

ZOCK, P.L., DE VRIES, J.H.M. AND KATAN, M.B. (1994). Impact of myristic acid versus palmitic acid on serum lipid and lipoprotein levels in healthy women and men. *Arterioscler. Thromb.* **14**, 567–575.

ZOPPI, G., ANDREOTTI, G., NJAI, D.M. AND GABURRO, D. (1972). Exocrine pancreas function in premature and full term neonates. *Pediatr. Res.* **6**, 880–886.