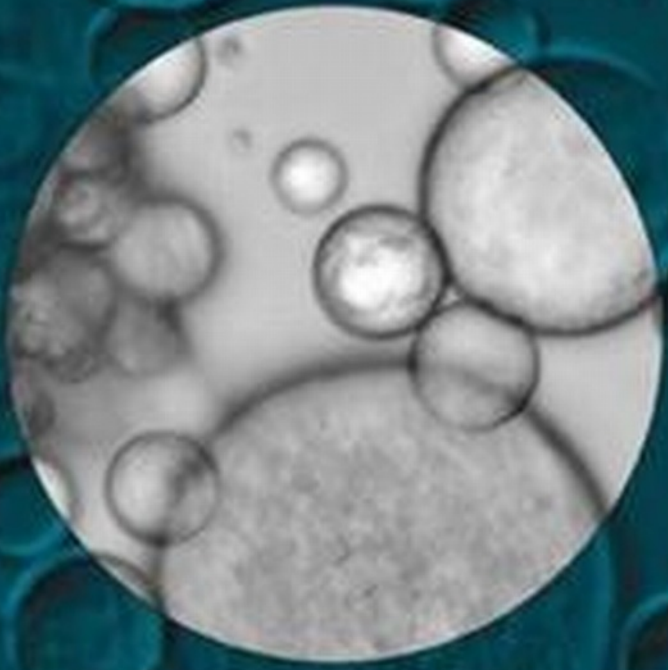
Single Cell Oils



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Single Cell Oils

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Dedication

To the memory of David Horrobin, 1939–2003, a scholar, a pioneer, and an inspiration to many.

Preface

Single cell oils (SCO) have come of age. They have become accepted biotechnological products fulfilling key roles in the supply of the major very long chain polyunsaturated fatty acids (PUFA), now known to be essential for infant nutrition and development. But their acknowledgment as being potential sources of oils and fats has been a slow process. Many critics in the early years of SCO doubted whether they could ever be produced at a reasonable price; even if they could, there were grave doubts as to whether SCO would be accepted by the general public. This was in spite of the "general public" having no apparent objection to consuming bacteria and yeasts as part of their everyday diet in the form of yogurts, cheeses, beers, and sourdough breads. When the product is good, the public will buy it; when the product is essential, the public will line up to buy it; and when our babies need the product, the line is likely to be a very long one indeed.

SCO are the edible oils extracted from micro-organisms—the single-celled entities that are at the bottom of the food chain. The best producers with the highest oil contents are various species of yeasts and fungi with several key algae also able to produce high levels of nutritionally important PUFA. Interest in SCO, as they have now become known, stretches back for over a century. Attempts have been made to harness the potential of various organisms, especially during the two world wars, in order to produce much needed oils and fats. Attempts have also been made to produce substitute materials for some of the major oilseed crops and even to produce a superior type of cocoa butter material. But it has been their potential to produce PUFA that has now galvanized the current interest in these SCO as oils rich highly desirable fatty acids essential for our well being and not readily available either from plants or animals.

This monograph has arisen from a symposium organized by David Kyle for the American Oil Chemists' Society in May 2003 that covered many of the ongoing projects in this area. It echoes two earlier conferences of the AOCS, the first in 1982 in Toronto and the second in Chicago in 1992, also organized by David Kyle. Over the intervening years, the position of SCO has become much more secure. Processes that were just "twinkles in the eye" in 1992 now exist as commercial realities; SCO production processes occur not only in the United States, but also in Europe, Japan, and China. Interest in them is widespread and the prospects of producing a complete range of PUFA is within our grasp. Whether the next decade or so will see SCO being overtaken by oils coming from genetically engineered plants, as has been predicted by some, will remain a tantalizing prospect. The future, as always, will be awaited with interest. In the meantime, SCO are here and available.

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January 2005

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Chapter 1

Single Cell Oils for the 21st Century

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Introduction

Single cell oils (SCO) might be defined as the edible oils obtainable from microorganisms and are similar in type and composition to those oils and fats from plants or animals. This chapter aims to provide an introductory overview to SCO and to show that the current interest in their production and use comes from a long history of interest in the exploitation of microorganisms as sources of oils and fats. Without these early endeavors, it is quite possible that none of the current commercial SCO products on the market would have been developed, since the basic understanding behind the exploitation of microbial oils would have delayed for several decades. The key events that led to the transition of microbial oils from being more or less academic curiosities 30 years ago to being important nutraceuticals included in infant formulas now were the overwhelming evidence of the dietary significance of very long chain, polyunsaturated fatty acids (PUFA) coupled with the realization that there is no adequate or safe source of them from plants or animals. What were originally unusual microorganisms have now turned out to be extraordinarily important, since these are the only realistic sources of these oils. The diversity of microorganisms is so great that it can almost be guaranteed that these current products will not be the last ones that will be launched in the 21st century as SCO.

The Early Years

There has been interest in microbial lipids for over 125 years (1) and in exploiting them as alternative sources of oils and fats for human consumption probably since the early years of the 20th century. Paul Lindner, working in Berlin, Germany, appears to have been the first person to develop a small-scale process to make a fat using a species of yeast then called *Endomyces vernalis* and currently known as *Trichosporon pullulans* (2,3). Work on the prospects of using microorganisms as a source of oils and fats continued to escalate during the first four decades of the last century with a number of groups in various countries studying not only the process of lipid biosynthesis but also the factors influencing its accumulation. These early endeavors into microbial oil production were reviewed in considerable depth by Woodbine (3) and

this review possibly remains the most thorough that is available covering world-wide developments of the subject from its very inception up to the mid-1950s.

The problem, though, was that the oils and fats produced by oleaginous species of yeasts and fungi (the groups of microbes that were the highest producers were called the "oleaginous" species) were not too different from the oils and fats obtainable from plant seeds. As these microorganisms had to be grown in culture medium that contained glucose or sucrose as a source of carbon, which was derived from agricultural crops, the cost of turning one agricultural commodity into another (i.e., turning sugar into oil) was never going to be economically feasible as the cost of sugar is never more than about a quarter of most of the commodity plant oils such as corn oil, soybean oil, and rapeseed (Canola) oil. Moreover, it is not a question of turning one ton of sugar into one ton of oil. Microorganisms are not that efficient; it takes about 5 tons of sugar to make one ton of oil. It can be appreciated that either some zero-cost carbon source or oils that exceed the prices of the usual commodity oils by a considerable margin must be found.

In spite of these obvious economic limitations, considerable work on the production of microbial oils took place from the 1920s up to the late 1950s. This laid some very important foundations to understand lipid production in microorganisms. In brief, it was established that:

The number of microorganisms capable of accumulating oil more than about 20% of their biomass weight was relatively small in comparison with the total number of species.

The oil-accumulating microorganisms were mainly species of yeast and fungi; few bacteria produced much extractable edible oil. The oil produced by these microorganisms was, like plant oils, mainly composed of triacylglycerols having component fatty acids (FA) that were, in almost every case, similar to what had already been recognized in plant oils.

Some algae were recognized that produced fairly high amounts of lipid, but this lipid tended to be more complex than those from the yeasts and fungi; they still contained the same FA that occurred in plant oils. Some PUFA were observed to be similar to those found in fish oils.

Oil accumulation in the oleaginous microorganisms could be increased by starving the cells of a supply of nitrogen—or a nutrient other than carbon. The cells responded to the deprivation of a key nutrient by entering into a lipid storage phase in which excess carbon, still present in the growth medium, was converted into storage lipid materials. If the cells were subsequently returned to a situation in which the missing nutrient was nitrogen available, the oil reserves could be mobilized and rechannelled into cellular materials. Lipid accumulation was a stress-induced response with the oil being an intracellular storage, reserve material.

A typical profile for the accumulation of lipid in an oleaginous microorganism is shown in Figure 1.1. This shows that lipid accumulation in a microbial cell only begins when nitrogen is exhausted from the medium. The medium therefore has to be formulated with a high C:N ratio to ensure that nitrogen is exhausted while other

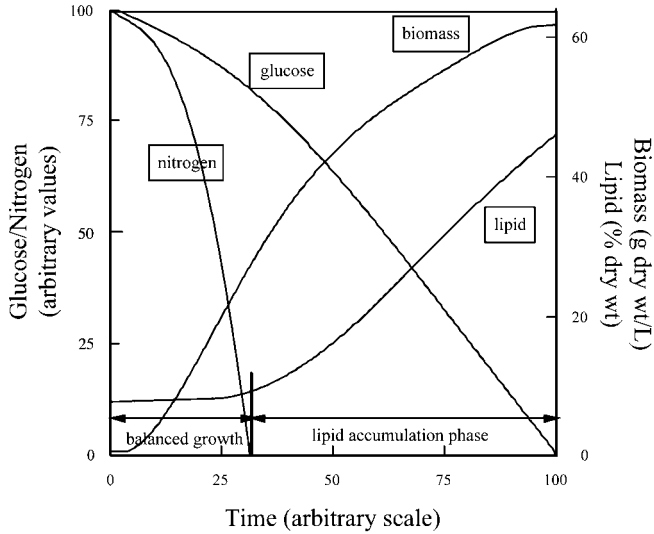

Fig. 1.1. Idealized representation of the process of lipid accumulation in an oleaginous microorganism. The composition of the culture medium is formulated so that the supply of nitrogen, which is usually an ammonium salt is growth limiting. After its exhaustion, cells do not multiply any further, but they continue to assimilate glucose (the usual carbon feedstock). This is then channelled into the synthesis of storage lipid (triacylglycerol) within the cells. The extent of lipid accumulation is dependent upon the individual microorganism—lipid contents may vary between 20 and 70% of the biomass.

nutrients, including carbon, remain in excess. In practice, this is about 40 to 50:1 (C:N) although the optimum ratio needs to be determined for each individual organism. To produce the greatest number of cells, the concentration of nitrogen and carbon may need to be increased while keeping them in the same proportion; this enables the balanced growth phase to continue until the maximum biomass density that the fermentor can sustain is reached before the lipid accumulation phase begins.

Although attempts were made in Germany during World War II to produce microbial fat to supplement the meager supplies that could be obtained from conventional sources (mainly animal fat with a little plant oil), these efforts were limited. However, some oil-rich biomass production was achieved with fungi. The fungi, mainly *Oidium lactis* (now *Geotrichum candidum*), was grown on waste lactose (from a cheese creamery) or agricultural waste material (4-7); this seems to have been fed mainly to army horses by being formed into bricks using hay and straw (4). Some may have been included in soups and sausages for human consumption, but it was mainly viewed as a protein supplement rather than a source of fat. Although feeding the oil-rich fungus to army horses may sound rather trivial, the German army during this period had up to one million horses to support and clearly using unconventional sources of feed material was considered entirely reasonable.

Development of efficient large-scale production of microbial oils was limited by the availability of appropriate large-scale fermentors necessary to produce the biomass (microbial cells) to high densities (over 50 g dry wt/L). Laboratory-scale fermentors were relatively unheard of up to the 1950s, and industrial-scale stirred tank fermenters were rare. This lack of technology was demonstrated by the UK having to transfer the technology for penicillin production in early 1940s (which had used static cultivation of *Penicillium chyrsogenum* in adapted hospital bed-pans), to the US which had the only accessible stirred tank bioreactors in the world. This lack of technology was a clear limitation not only to microbial oil production but to almost all other microbial products that needed aerated, submerged cultivation systems. Some fermentors existed in many countries to produce beer and related materials, but these were for anaerobic production of microbial products and had no facilities for aeration or stirring. Moreover, most were open vessels and therefore were prone to airborne contamination.

The major stimulus to develop large-scale fermentation technology, and from it for the production of laboratory-scale fermentor units, was probably the advent of single cell protein (SCP) production that began in the late 1950s. Several petroleum companies, but principally BP Ltd of the UK, began to explore the conversion of *n*-alkanes, unwanted waste materials from the initial phase of fractionating petroleum oil, into edible biomass. Yeasts (especially *Yarrowia lipolytica*) were found that could grow rapidly on the alkanes, but to achieve optimal conversion stirred and aerated fermenters were essential. The ensuing biomass was rich in protein (about 50% w/w) and proved to be a useful major feed material for animals. As the manufacturers felt a little uneasy about describing their product as "microbial protein," the name SCP was coined as an appropriate euphemism to disguise the origins of the material.

This period ended because of unfavorable economics in 1975 with the price escalation of crude petroleum oil and the maintenance of the low price of soybean meal—the major competitor of the SCP. At the end of this period, the world had developed systems for submerged microbial cultivation to an unparalleled degree. Biotechnology had arrived! And not just for SCP production; production of antibiotics, amino acids, and organic acids such as citric acid, were now using sophisticated, stirred tank fermentation technology which had replaced cultivation of microorganisms in static cultures that primarily used shallow tray systems.

With the new technology becoming widely available (not forgetting the availability of laboratory-scale fermentors at a reasonable cost to allow research to be carried out at the 1-2 L level) interest in producing microbial oils once more re-emerged in the mid-1960s (8,9). However, enthusiasm for producing such products had largely waned since plant seed oils were now extremely inexpensive and there seemed little if any prospect of producing oils from other sources that could rival their price. There were, though, some prospects of producing some microbial oils (10-12) that were not readily available from conventional plant sources, but these ideas were still embryonic and lacked focus because the market for such materials was very uncertain. It is pertinent to point out that the examination of microorganisms carried out by Robert Shaw

in the early 1960s was focused on identifying possible sources of arachidonic acid (ARA; 20:4n-6)—not for use in human nutrition (for which nothing was known at that point) but as a chicken-flavor material! Only after the work had been done was it realized that chicken flavor was not due to ARA but to some entirely unrelated compound. The work of Shaw, however, proved invaluable for identifying microorganisms that might be used for the production of various long chain PUFA.

The other main development that occurred in the early 1960s and was of considerable importance for the study of microbial oils, was the development of gas chromatography (13,14). Previously, FA analysis had been laborious and tedious and also required relatively large amounts of material. Gas chromatography altered all this; almost instantly one could analyze a number of oils and fats for their component FA and, moreover, use just milligram amounts of material. The stage was therefore set for a reexamination of microorganisms as potential sources of oils and fats; this can be seen from the seminal work of Bob Shaw mentioned previously and carried out from about 1960 to 1964.

Developments in the Last Quarter of the 20th Century

Although work in the author's laboratory (15-18) had been able to consolidate the mechanism of oil accumulation in yeasts being grown in laboratory fermentors using both batch and continuous fermentations and to confirm the approximate conversion efficiency of the starting substrate (glucose) to the product (triacylglycerol oil), there was, however, no clear target of which oil would be appropriate to consider for development. It was then brought to the author's attention that there might be a small niche market for an oil rich in γ-linolenic acid (GLA, 18:3n-6).

A Process for GLA Production

In the mid-1970s, GLA was only available as a minor component (about 9% of the total FA) of evening primrose oil (*Oenothera biennsis*), but nvertheless this oil was considered efficacious to relieve many symptoms and even for the treatment of multiple sclerosis—a claim that has long since been discounted—by virtue of its content of GLA. At the time, evening primrose oil commanded a price of about $50 per kg when most commodity plant seed oils were fetching less than a hundredth of this. Instantly, the prospects of a commercially viable SCO were presented since it was known that there were microorganisms that synthesized GLA, and the work of Shaw (10-12) had established its consistent occurrence in a group of lower fungi known as the Zygomycetes.

Research carried out in the author's laboratory established that one member of this group was entirely suitable for producing an oil rich in GLA using large-scale submerged fermentation technology and commercialization of the process then followed with the first oil being produced in 1985 (19).

The first SCO was thus produced using *Mucor circinelloides* grown in large-scale fermentors of 220 m^3 (55000 US gallons). It was run by J. & E. Sturge Ltd at Selby,

North Yorkshire, UK, who normally used their skills in fermentation technology to produce citric acid using another fungus, *Aspergillus niger*. The oil was sold under the trade name of Oil of Javanicus and also as GLA-Forte that was used by one retailer of the oil. It achieved some limited penetration of the over-the-counter, food supplement market. By the time the process closed down in 1990, primarily due to a change in ownership of the company (to Rhone-Poulenc Ltd), falling prices in evening primrose oil, and the advent of borage oil as a cheaper alternative source of GLA, about 50 tons of material had been produced. Each fermentor run produced about 10 tons of biomass from which about 2 to 2.5 tons of oil could be extracted (see Chapter 13). A more detailed account of the SCO-GLA process is available (19).

Although it was superior to evening primrose oil in all respects, higher content of GLA (Table 1.1), higher stability to oxidation, absence of high levels of competing FA such as linoleic acid, lower content of herbicide and pesticide residues, the fungal oil had difficulty in being sold to a public (mainly in the UK and some other European countries) that wanted evening primrose oil. Something that was superior to evening primrose oil but was not called "evening primrose oil" was viewed with suspicion even though marketing publicity carefully eschewed mentioning the microbial origins of Oil of Javanicus.

Although this first SCO failed to bring in a reasonable profit for the producers, nevertheless it was a significant milestone in the development of SCO. Its arrival encouraged other companies in other countries to explore the possibilities of using microorganisms as sources of similar and even more expensive oils and fats. Targeting of potential oils for niche markets was, however, still critical. A process related to the GLA-SCO process in the UK was developed in Japan by Idemitsu Kosan Co. Ltd, Tokyo, Japan using *Mortierella isabellina* and possibly also *Mort. ramanniana* (20). The oils produced were, however, much lower in GLA content than the oil produced by *Mucor circinelloides* (Table 1.1) though each fungus had about

TABLE 1.1.
Fatty Acid Profiles of Fungi and Plants Used Commercially for γ-Linolenic Acid Production.

		Relative % (w/w) of Major Fatty Acids								
	Oil Content (% w/w)	16:0	16:1	18:0	18:1	18:2 (n-6)	18:3 (n-6) GLA	18:3 (n-3)	20:1	22:1
Mucor circinelloides[a]	25	22	1	6	40	11	18	—	—	—
Mortierella isabellina[b]	~50	27	1	6	44	12	8	—	0.4	—
Mortierella ramanniana[b]	~40	24	—	5	51	10	10	—	—	—
Evening primrose	16	6	—	2	8	75	8–10	0.2	0.2	—
Borage	30	10	—	4	16	40	22	0.5	4.5	2.5
Blackcurrant	30	6	—	1	10	48	17	13	—	—

[a]Oil of Javanicus, citric acid produced by *Aspergillus niger*.
[b]Production organisms used by Idemitsu Co. Ltd, Japan. Oil contents of cells uncertain but approximate levels indicated.

twice the oil content of the *Mucor*. Sales of these oils to the Japanese domestic market began in 1988 though it is not known how much was sold or even if the process(es) are still extant; if the processes have now ceased, as seems likely, again it is uncertain when this occurred.

A Process for a Cocoa Butter Equivalent Fat

Some interest was developed in the early 1980s with the possible production of a cocoa butter equivalent (CBE) fat using yeasts. Yeasts, unlike many molds and fungi, tend to produce only limited amounts of PUFA and some strains can have relatively high contents of stearic acid (18:0). For a successful CBE, it is necessary to have a oil or fat produced that has roughly equivalent amounts of stearate, oleate, and palmitate all accommodated on the same triacylglycerol molecule preferably as *sn*-1 stearoyl, *sn*-2 oleoyl, *sn*-3 palmitoyl glycerol (Table 1.2). The main problem to achieve this goal was to increase the rather low content of stearic acid in yeast fat up to at least 25%. This was initially attained using an inhibitor of the Δ9-desaturase that converts stearic acid into oleic acid (21). However, the inhibitor used, stearidonic acid, was found to be more expensive to use than could be tolerated by required price of the final product. Instead, mutants of a yeast, *Candida curvata* (now *Cryptococcus curvatum*) were produced that had altered activities of this desaturase and thus produced the same type of product without having to use an expensive inhibitor (22) (Table 1.2). The mutants that were produced were not entirely stable, however, when used in large-scale fermentors; it was preferred to use the original, wild-type yeast, which had already a higher natural level of stearic acid than most other yeasts as a possible production organism (23,24). The key procedure used to increase the level of stearic acid was to use a very low aeration rate so that the desaturases were limited in their activities by oxygen availability, which is a co-substrate for their activity.

TABLE 1.2
Fatty Acid Profiles of Cocoa Butter Equivalent (CBE) Single Cell Oils (SCO): Microbial Oils Used as a CBE Compared with Cocoa Butter.

	Relative % (w/w) of major fatty acids					
	16:0	18:0	18:1	18:2	18:3 (n-3)	24:0
Cryptococcus curvatus Wt[a]	30	15	45	5	0.5	2
C. curvatus Nz[b]	18	24	48	3	1	2
C. curvatus R26-20[c]	15	47	25	8	2	—
C. curvatus R25-75[c]	33	25	33	7	1	—
C. curvatus F33.10[c]	24	31	30	6	—	4
Yeast isolate K7-2[d]	26	25	38	6	1	1
Cocoa butter	23–30	32–37	30–37	2-4	—	—

[a]W, wild type yeast (original strain).
[b]*NZ*, strain used in New Zealand. See Davies (23).
[c]Mutant strains produced with partial deletions of Δ9-desaturase. See Smit *et al.* (22)
[d]Isolated in New Zealand. See Davies (23,24).

In spite of achieving a good quality CBE (Table 1.2) that could be incorporated into chocolate at the permitted level of 5% of the total fat, giving improved characteristics over the use of a conventional plant-derived CBE (R.J. Davies, personal communication), the yeast process was abandoned as not being sufficiently cost-effective. The cost of cocoa butter, which had been up to about $8000 per ton when the research work had begun, had fallen by the late 1980s to about $3000 per ton; since a CBE could only fetch about 60% of this price, this left insufficient profit for the process to proceed beyond the pilot-scale level and an initial, one-off large-scale run at 250 m3 (23). This was in spite of the process using virtually zero-cost lactose as feedstock with the lactose arising from the cheese creamery processes in New Zealand where there is so much of it that there are severe problems in ensuring its environmentally-friendly disposal!

Also taken into consideration when deciding to abandon this yeast CBE-SCO project was the uncertainty about the chocolate industry using the product even in confectionery products (e.g., for cakes, toppings, etc.) rather than in chocolate for direct consumption. Unease at using a "microbial fat" in chocolate products that depend very much on marketing images for high sales was a telling factor. Thus, with its market take-up being uncertain, the presence of adequate, alternative sources of other CBE, namely from palm oil fractionation, and the apparent low profitability of the microbial process, another SCO program then was terminated.

SCO for the 21st Century

The Quest for a Docosahexaenoic Acid-Rich SCO

Having established that microorganisms could produce high quality oils and fats—though admittedly at a price—it was then a question of identifying which, if any, possible market might be exploited by these materials. Top consideration had to be given to oils that would be appropriate for human consumption rather than for animals, since these would be the markets able to command the highest prices as had already been seen with the GLA-SCO. At the same time, oils that could not be readily obtained from plant or animal sources would give aditional advantage to a microbial route of production as the ensuing oil would then be free of serious competition. With these general considerations in mind, the work on the nutritional benefits and effects of the very long chain PUFA found in fish oil was of major importance.

There had been a steady investigation of the possible dietary benefits of fish oil since the pioneering work of Sinclair in the 1940s (25). However, the major findings that received international recognition arose from reports from Danish scientists investigating the reasons why cardiovascular problems seemed nonexistent, or at least significantly less, in Greenland Eskimos compared to other populations in spite of the very high intake of fat by the Eskimos (26). A low incidence of heart disease in other fish-eating populations of Norwegians and Japanese also helped to focus attention on the importance of docosahexaenoic acid (DHA; 22:6n-3) and eicosapentaenoic acid (EPA; 20:5n-3), being the two major PUFA of fish oils. By the 1980s, the importance

of both EPA and DHA for human nutrition was established and then, in the 1990s, the particular beneficial effects of giving DHA during pregnancy and for the nutrition of premature and newly-born, full-term babies began to appear (26,27). The presence of DHA and ARA in mother's milk and their occurrence as the major FA of brain lipids and the retinal membrane lipids reinforced the concept that it would be highly beneficial if both these FA could be included in the diet of pregnant women and in infant formulas designed for the neonatal baby. A more complete account of the nutritional advantages of DHA and ARA is covered expertly in Chapter 12.

Since it was DHA rather than EPA that was considered important, this meant that fish oils were not entirely satisfactory sources because all these oils contained both FA in roughly equal proportions (26); EPA was not, however, a "neutral" material that could be taken along with DHA. It appeared to metabolically interfere with the efficacy of DHA uptake and its incorporation into brain and retinal lipids and thus was counter-indicated (28). Tuna oil, though, appeared to be an exception to most other fish oils; it has a DHA to EPA ratio of 4:1 (26) which is about the same as occurs in mother's milk. But tuna oil was clearly in short, if not diminishing, supply and, in any case it did still have some EPA. The only solution that seemed appropriate was to embark on a very expensive process to fractionate DHA from fish oil. This would require several steps culminating in the use of preparative level high performance liquid chromatography (HPLC), which, by its very nature, was prohibitively expensive. No other source of DHA seemed apparent to nutritionists during the early 1990s.

Nutritionists, however, are not microbiologists and tend not to bother about microbial lipids or to know much about their composition except for recognizing that some marine microorganisms do contain DHA but usually with EPA in association. It did not seem apparent to any nutritionist in the late 1980s that microorganisms could be the key to providing a supply of DHA. It took someone who was aware of both the need for a good supply of DHA-rich oil and, simultaneously, had a knowledge of the FA composition of key microorganisms to put, literally, two and two together and identify a potential microbial source of DHA. This was a major breakthrough and was pioneered by David Kyle and by the launch of his company, Martek Ltd, in the late 1980s that focused exclusively on developing a process using *Crypthecodinium cohnii* as the organism of choice for DHA production. *Crypthecodinium cohnii* was, though, already well-known as a producer of an oil rich in DHA and of no other PUFA (29,30) but it was not apparent that it could be grown in very large scale fermentors to produce sufficient biomass to warrant considering it as a commercial source of oil. Kyle and his colleagues, in a remarkably short period of time, demonstrated that this was feasible and they then went on to produce this oil which has since had a major impact on the infant nutrition market. A detailed account of the current process for producing DHASCOTM is given in Chapter 6.

An ARA-Rich SCO

DHA, however, as indicated previously, was not the only FA that appeared to be important in infant nutrition. The other FA was ARA (28). By a happy coincidence, a

microbial source of ARA was already known through the work of Shimizu in Japan (Chapter 2) using the Zygomycetes fungus, *Mortierella alpina* (31,32). However, the use of this oil for infant nutrition had not been considered and, thus, the opportunity of exploiting this technology independently of the Japanese work was then undertaken, again by Martek.

Martek, it has to be said, was the only company that recognized what was needed by the infant formula market, by way of very long chain-length polyunsaturated FA (VL-PUFA), and knew how to obtain them. The foresight of the company was the recognition that both DHA and ARA could have huge markets even if only, say, 10% of all newly born babies were fed on enriched infant formulas and even if only 0.1% of the weight of the formula might be DHA/ARA. Multiply 0.01% of all infant formulas that are produced in the USA and Europe (not to mention in the 100+ other countries in which the product is now sold) and the potential of the SCO for this market can be quickly appreciated.

Microbial production of oils rich in both these FA are now the main SCO in current production. Both processes were developed by Martek, though the one for ARA was further developed by DSM (Chapter 5) working under license from Martek. Both processes began at a commercial level in the 1990s (33,34), and both are set to continue their expansion during the remaining years of this decade. In all probability, they will continue to dominate the market for both DHA and ARA for some time to come as it is highly unlikely that the demand for these VL-PUFA will diminish. Indeed, all the indications are that the demand for both FA will continue to grow until possibly there will be no infant formulas being produced in Western countries that will be without both materials. The only change in this is the rather unlikely event of a significantly higher proportion of mothers choosing to breast-feed their babies rather than opting for formula-feeding.

The FA profiles of the commercial SCO are given in Table 1.3.

Other Sources of PUFA-SCO

DHA-Rich Oils. Not unexpectedly, once the DHA-SCO and ARA-SCO oils were announced, other possible microbial sources of these materials were examined. An account is given in Chapter 3 of the process developed by OmegaTech Ltd, Boulder CO, to produce an oil rich in DHA using a species of *Schizochytrium* (35). Briefly, the oil produced was not a "DHA-only" oil but had about 20% of the DHA content as docosapentaenoic acid (DPA; 22:5n-6). This latter FA, although not of the same n-3 family of FA as DHA, is metabolically neutral and does not detract from the efficacy of uptake of DHA into key brain lipids; it does not add to the DHA content of the oil and, to this extent diminishes the overall efficiency of DHA production in the organism. However, by the time this process was fully launched, the market for a DHA-only oil had been established by the *Crypthecodinium* oil and this has proved to be an unimpeachable position. The *Schizochytrium* oil, nevertheless, looks likely to be less expensvie than the former oil perhaps being half or even less the price as the organism grows about four times faster and also to very high cell

TABLE 1.3
Fatty Acid Profiles (Given as Rel. % w/w) of SCO in Current Production[a]

A. Arachidonic Acid-SCO Processes Using *Mortierella alpina strains*

	14:0	16:0	18:0	18:1	18:2	18:3 (n-6)	20:3 (n-6)	20:4 (n-6)	22:0	24:0
DSM process[b]	0.4	8	11	14	7	4	4	49	—	1
Wuhan Alking process[c]	0.2	6.3	2.2	3.7	4.0	1.6	—	70.2	2.7	5.3

B. Docosahexaenoic Acid -SCO processes

	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3 (n-3)	20:3 (n-6)	22:5 (n-6)	22:6 (n-3)
Martek process[d] (DHASCOTM)	4	20	18	2	0.4	15	0.6	—	—	—	39
OmegaTech process[e] (DHASCO-S)	—	13	29	12	1	1	2	3	1	12	25
Nutrinova process[f] (DHActive)	—	3	31	—	1	—	—	—	—	11	45

[a]For other abbreviations see Table 1.2.
[b]See chapter 13.
[c] See Yuan *et al.* (43).
[d]Uses *Crypthecodinium cohnii*, see Chapters 6 and 13.
[e]Uses *Schizochytruim* sp., see Chapters 3 and 13.
[f]Uses *Ulkenia* sp., see Chapter 7.

densities—cell dry weight values of over 200 g/L, attained after 72 hours' growth, have been claimed (36).

The marketing (but not production) of the *Schizochytrium* oil, which was originally known as DHAGold but is now named as DHASCO-S, is complicated by OmegaTech now being owned by Martek. Thus the senior company can chose to preserve the infant formula market for its own *Crypthecodinium* oil while exploiting other opportunities for the sale of the *Schizochytrium* oil. Such markets could well include feeding of farmed fish. Currently about 5 tons of fishmeal are needed to bring one ton of fish to maturity in these fish-farms. Clearly, this is nonsustainable and alternatives to fishmeal are now actively being sought. Since the key ingredient of fish meal for growth and development, especially in the very earliest stages of fish growth, are the VL-PUFA, then an alternative source of DHA would be extremely attractive. Although the costs of producing *Schizochytrium* biomass (for fish feeding one need not extract the oil but, instead, the whole biomass can be used) are considerably less than producing *Crypthecodinium* biomass, it would still appear to be more costly (possibly double) than fishmeal itself. Nevertheless, it is a sustainable source of DHA. If it ultimately turns out not to be too prohibitive in price, governments or regulatory agencies may then chose to ban fish meal, or at least place a moratorium on its use, in favor of a sustainable, alternative source.

A further reason for a move away from using fishmeal for fish feeding is the presence in fishmeals of various residues of man-made pesticides that have entered

the world's oceans and seas. These include dioxins and polychlorobiphenyls (PCB) as well as organo-heavy metals, including mercury compounds. Already the presence of such materials is too high to allow fish oils to be given as dietary supplements to infants in the USA.

Further markets for SCO are also likely to be developed for other food uses and, for which, either the oil itself or the biomass could be used. We have already seen the incorporation of *Schizochytrium* biomass into poultry feed to bring about DHA-enriched eggs, which have been a minor marketing success (37). DHA-enriched milk and milk-derived products (cheeses, yoghurts, etc.) and other food products are obvious extensions of this concept. It may be expected over the next decade or two that there will be a growing appreciation of the need for PUFA, such as DHA and perhaps ARA, in adult nutrition in addition to their use in infant foods. The development of a whole range of DHA-supplemented foods—from margarines to salad dressings—is then entirely feasible. The use of oils or biomass from organisms, such as *Schizochytrium*, is then bound to rise, and rise quite sharply, should these predictions be fulfilled.

It is also evident that further microbial sources of DHA are already being developed and considered as additional commercial sources of DHA-rich oils and DHA-rich biomasses to meet these expected increases in the market size for PUFA. Possible processes using marine organisms referred to variously as *Ulkenia* or *Labyranthula* are under development in Japan (38,39) and in Germany. The latter process is reviewed in Chapter 7. The organisms being used are similar in a number of features to *Schizochytrium* spp. (40,41) and their oils, like that of DHASCO-S, always contain a significant proportion of DPA (42) (Table 1.3) which further emphasizes the similarity of this group of organisms. Commercial establishment of other, alternative processes for DHA production will clearly benefit the public since this will give both choice and a competitive price for the product.

ARA-Rich Oils. Alternative microbial sources of ARA are also being sought. Already it is known that there is a process for ARA production in China, operated by Wuhan Alking Bioengineering Co. Ltd, using a new strain of *Mortierella alpina* (43). This process appears to operate at the 50-100 ton level (50,000–100,000 L). Work also appears to be ongoing to identify new organisms of interest for ARA production: a new strain of *Mortierella alliacea* has been reported with contents of ARA similar to those found in *M. alpina* of over 40% (44), and recent work (reviewed in Chapter 4) has found a new phototrophic algae, *Parietochloris incisa,* that has the highest content of ARA of any phototrophically grown alga at over 40% of the total FA.

The overall activity in these areas to identify new, and possibly, improved sources of DHA and ARA implies considerable economic potential in these processes. The lucrative nature of the markets will therefore continue to attract further interest from established biotechnology companies, and perhaps even pharmaceutical companies, all wishing for a share of the revenue.

PUFA-SCO for Clinical Applications. Clinical applications of the VL-PUFA seem, at the moment, to be restricted to the use of EPA rather than DHA or ARA, which are now regarded as nutraceuticals or dietary supplements. Currently EPA is produced expensively by fish oil fractionation and chromatography, though possible microbial sources of it are under active consideration in a number of laboratories around the world (Chapter 10). The potential market size for EPA is difficult to estimate since applications of this PUFA seek to alleviate or cure various illnesses, including schizophrenia, bipolar disorder, certain cancers, Alzheimer's disease, and atherosclerosis, which are usually treated by expensive pharmaceutical drugs. It is worth pointing out that pharmaceutical companies have a vested interest in maintaining the status quo being the sole providers of the expensive medications for the treatment of these illnesses and disorders. Pharmaceutical companies will not encourage clinical and medical practitioners to prescribe, or suggest consumption of, a simple, over-the-counter FA that, although expensive, will nevertheless be much cheaper than a pharmaceutical drug.

There is no doubt that EPA is a very useful anti-inflammatory compound and can be given safely to many types of patients (Chapter 10). Nevertheless problems about its source remain. Fish oils, for reasons discussed previously, are unlikely to be a satisfactory long-term source of EPA; for this reason alone alternative microbial sources would seem to be the preferred option. At the moment these sources would appear to be photosynthetic algae (Chapter 10), though it would seem quite likely that microorganisms could be found that would be able to grow heterotrophically and thus parallel the situation with *C. cohnii* and other organisms being used for DHA production. Heterotrophic cultivations of microorganisms, although requiring a fixed carbon source and more expensive equipment, has a much higher productivity than phototrophic cultures that more than offsets these disadvantages. The problems in identifying an appropriate source of EPA should not be underestimated, since researchers have been trying to identify such a source for at least the past 5 years. It should though be noted that when *Mortierella alpina* is grown at a low temperature and supplemented with α-linolenic acid (18:3n-3) can produce EPA instead of ARA (Chapter 2). However, the process would appear to require a lengthy cultivation period (45) thereby increasing the costs of the oil substantially.

Other PUFA-SCO for clinical use, and perhaps for dealing with specific metabolic disorders, await market opportunities. Prospects of producing a variety of other PUFA, besides DHA, ARA, and EPA, are discussed in Chapter 2 in which various mutants of *Mort. alpina* have been produced that synthesize useful amounts of stearidonic acid (18:4,n-3), dihomo-γ-linolenic acid (20:3n-6), eicosatrienoic acid (also known as Mead acid; 20:3n-9), and eicosatetraenoic acid (20:4n-3). Of these, only stearidonic acid can be obtained from a plant source (*Echium*). In addition to these FA, DPA which occurs in the DHA-rich oils from *Schizochytrium* spp., is thought to be produced by Nagase-Suntory Co. Ltd in Japan. Whether this is produced as a by-product from the fractionation of the *Schizochytrium* oil or is produced using a specific organism, such as the novel labyrinthulid isolate that was recently reported to pro-