Single Cell Oil

Edited by R.S. Moreton

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Cadbury Schweppes Group Research,
Lord Zuckerman Research Centre,
University of Reading
Reading, RG6 2LA, Berkshire, U.K.

Present address: Nestec Ltd.,
Nestlé Research Centre,
P.O. Box 353,
CH-1800 Vevey, Switzerland.

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FOREWORD

There has been intermittent interest in production of lipids by unconventional routes such as fermentation and plant cell culture for at least the last 50 years. These reports have appeared principally in the technical literature scattered amongst a wide range of journals covering specialised areas.

Technology for the production of such lipids is now well established and proven, and given an economic incentive these products could be on the market place within a few years.

In this book, we have attempted to collect data from these various sources on the biochemistry, technology and economics of such processes and present them in such a form as to allow readers to draw their own conclusions regarding the applicability of this technology to their own circumstances.

There is no doubt that at the present time there is little prospect of oils and fats produced by such processes competing with conventional sources of edible oils for economic reasons. There are some situations, however, where conventional economics do not apply, for example with waste materials o where the political situations imposes levies against imports. Unusual lipids, such as gamma linolenic acid, and probably others, can undoubtedly be produced economically by fermentation today.

R. S. Moreton.

March 1987.

CONTRIBUTORS

Chapter 1

R. S. Moreton Cadbury-Schweppes Group Research,
Lord Zuckerman Research Centre,
University of Reading,
Reading, Berkshire, RG6 2LA, U.K.

Present address: Nestec Ltd.,
Nestlé Research Centre,
P.O. Box 353,
CH-1800 Vevey, Switzerland.

Chapter 2

C. Ratledge Department of Biochemistry,
University of Hull,
Hull, HU6 7RX, U.K.

Chapter 3

A. H. Scragg &
R. R. Leathers Wolfson Institute of Biotechnology,
University of Sheffield,
Sheffield, S10 2TN, U.K.

Chapter 4

R. J. Davies Industrial Processing Division,
Department of Scientific and
Industrial Research,
Private Bag,
Petone, New Zealand.

Chapter 5

E. G. Hammond &
R. A. Glatz College of Agriculture,
Department of Food Technology,
Iowa State University,
Ames, Iowa, 50011-090, U.S.A.

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1 PHYSIOLOGY OF LIPID ACCUMULATING YEASTS

R. S. Moreton

1. INTRODUCTION

The term 'Single Cell Oil' (SCO) was probably coined by one of the contributors to this volume (Thorpe & Ratledge, 1972) comparing the production of edible protein in the form of micro-organisms (Single Cell Protein - SCP) with the production of edible oil from micro-organisms, hence Single Cell Oil. This volume will concentrate on the possibility of using SCO to replace edible oils currently derived from plants which form the bulk of commercially traded edible oils. Many areas of physiology, technology, analysis and biochemistry are common to the production of both edible and non-edible oils or those with specialised applications and passing reference will be made to other areas where there are valid general or specific points to be made.

At the time of writing there is probably only one microbial oil process in production in the world, in the USSR, and little is known of the organism, technology or purpose for which the oil is used. Informed guesswork suggests that the organism is one of the red yeasts (*Rhodotorula* or *Rhodosporidium*) and that the oil is a by-product of an SCP process based on a hydrocarbon feedstock similar to the British Petroleum SCP process. If so, the oil is probably not of an edible grade, and is extracted along with residual substrate which is probably the main purpose of the extraction process. This strictly is not an SCO process in the terms of the above definition, therefore.

It has recently been announced that a UK company (Sturge Biochemicals) is constructing a plant to produce a lipid rich in gamma linolenic acid (GLA; 6,9,12 octadecatrienoic acid) by a fungal fermentation process (Sinden, 1987). This relatively rare analogue of the more common alpha linolenic acid (9,12,15 octadecatrienoic acid) is found in the seed oils of evening primrose, borage and blackcurrants and incorporated into capsules usually sold through healthfood outlets claiming beneficial effects in the treatment of stress related conditions such as eczema and pre-menstrual tension and a variety of other conditions. This is probably the first true commercial SCO process.

The nomenclature of lipids and fatty acids causes problems with those unfamiliar with the area, and it is not the purpose of this review to rectify this. There are several specialist texts which should be consulted if necessary, such as those by Gurr and James (1980), Gunstone, Harwood and Padley (1986) and Harwood and Russell (1984). It will suffice to say here that the general term lipid includes both fats and oils, the difference between these two being that fats are solid at ambient temperatures and oils liquid, basically as a result of their relative content of saturated or unsaturated fatty acids, saturated fats having a higher melting point than unsaturated oils. Most common commercial oils and fats are composed of 16 and 18 carbon straight chain fatty acids with varying degrees of unsaturation. The 16 carbon fatty acid is usually the saturated acid, palmitic, whereas the 18 carbon acids can be saturated (stearic), mono-unsaturated (oleic), di-unsaturated (linoleic) or tri-unsaturated (linolenic), these latter two being the most common polyunsaturated fatty acids (PUFAs). In shorthand form these acids are designated 16:0, 18:0, 18:1, 18:2 and 18:3, the first figure referring to the number of carbon atoms and the second to the number of double bonds. The terminology used previously to describe the alpha and gamma isomers of linoleic acid is the more rigorous systematic chemical name, the numbers indicating the positions of the double bonds relative to the terminal carboxyl group. Under physiological conditions, fatty acids do not exist as the free acid within cells or micro-organisms but most commonly as tri-esters of glycerol (triglycerides or triacylglycerols). There is a tendency to forget this in the literature and equate fatty acid composition with the lipid composition. The reason for this is that analytical techniques to determine the fatty acid composition of a lipid are relatively simple whereas those necessary to determine the structure of triglycerides are more complicated and require considerable experience before reliable results can be obtained (see Chapter 5).

Microbial lipids, in addition to the more common even numbered 16 and 18 carbon fatty acids described above, can contain numerous relatively unusual fatty acids, odd numbered, branched, hydroxy, cyclopropane or ether fatty acids amongst others, as well as short and long chain fatty acids and waxes. As well as neutral, non-polar triglycerides, other materials will be extracted by the procedures described in Chapter 5, particularly if using such powerful solvents as chloroform/methanol mixtures, most notably polar phospholipids and glycolipids. These are removed in the washing stage of the Bligh and Dyer modification of the Folch method described in Chapter 5, but beware of reports which categorise all material soluble in organic solvents as "lipid".

Some of these polar and other lipids have interesting properties, but as yet most of them are oddities seeking an application which could make them worth exploiting. As far as this book is concerned, "lipid" and triglyceride are synonymous since commercial fats and oils are essentially pure triglycerides, most other material having been removed in the refining process.

The field of microbial lipids in general has been well reviewed, most recently by Ratledge (1982), and it is not intended to reiterate the ground covered previously, except where necessary to illustrate a point or to put work in perspective. The emphasis of this introductory chapter will be on recent work, mainly over the last five years or so, with relevance to the large scale production of lipid producing (oleaginous) micro-organisms.

Before discussing microbial oils, however, a brief description of the world oils and fats market will enable the reader to put the mainly hypothetical proposals advanced in this volume into context relative to the very real world of commodity trading.

2. The world oils and fats business

World trade in oils and fats is substantial in both volume and financial terms, about 60 x 10^6 metric tonnes (MT) annually worth at

Table 1. Production and Price data for the principal commercial oils and fats

Oil or fat	Annual world production, metric tonnes x 10^6 average 1959-63	1964-68	1980-81	Current (estimate)	Current Price[2] US$/ metric tonne
Soya oil	3.944	7.681	12.431	13.930	392
Palm oil	0.614	0.639	5.034	8.680	254
Sunflower oil	2.559	3.630	4.724	6.430	413
Rapeseed oil	1.681	1.765	3.863	6.330	316
Cottonseed oil	3.550	3.693	3.247	3.440	389
Groundnut oil	4.457	5.048	3.110	3.500	608
Coconut oil	2.065	2.174	3.325	3.140	233
Olive oil	1.4.4	1.299	1.840	1.650	1,872
Palmkernel oil	0.869	0.508	0.696	1.190	350
Corn oil	0.199	0.236	0.518	0.725	560
Cocoa butter[1]	0.586	0.602	-	0.800	4,500
Linseed oil	1.115	1.054	0.696	0.690	800
Castor oil	0.258	0.340	0.396	0.345	1,870
Tung oil	0.109	0.132	0.090	-	925
Lard	-	-	3.827	-	497
Butterfat	-	-	4.972	5.390	-
Fish oil	-	-	1.222	1.330	340

1. Little cocoa butter is actually traded on the open market. These figures represent production of cocoa butter as a component of traded quantities of cocoa beans.
2. For illustrative purposes only. Prices are subject to rapid changes and can be quite different around the world.

Data from several sources, principally various issues of the Journal of the American Oil Chemists Society.

least \$150 x 10^9. Most of the major oils and fats are shown in Table 1, together with annual world production of each product for the 1984/85 trading year and historical data for production in 1980/81, and averages for 1964-68, and 1951-63. The prices shown are typical European prices in April 1985. Of the annual world production, about 80% is destined for human food use in some form such as margarine, cooking oil, salad oil, shortenings for bakery products, etc. The remainder has various uses such as soap and specialised technical applications such as lubricants, paints and varnishes. Paints and varnishes use the non-edible so called "drying oils", linseed and tung, whereas castor oil is mainly used as a lubricant. Excluding butter, over 90% of oils and fats are of vegetable origin, with soya oil having a 22% share of the marketplace followed by palm, sunflower and rapeseed with about 10% each.

Despite the huge growth in production over the last 25 years, there is still an overall shortfall in the world supply of these commodities, with no sign of supply being able to meet potential demand. The USSR and India each imported about 1.5 x 10^6 MT of edible oils in 1984/85, the total volume of the export trade from all countries being about 15 x 10^6 MT in that year. The trade is dominated by soya oil from the USA which produces about 10.1 x 10^6 MT annually, 76% of the world supply of this product and 17% of the total world production of oils and fats. The price of soyabeans and soya oil, therefore, effectively controls the price of most of the other vegetable oils, since to a large extent these commodities can be substituted one for another in many products such as margarine, vegetable shortenings or salad oils. Only where there is a specific property unique to some particular oil can it command a price substantially different from the level set by soya oil.

Changes in production of various oils are often not simply the result of market forces in this area. For instance, production of corn (maize) oil has increased dramatically due to increased production of hydrolysed corn syrups as a replacement for sucrose as a sweetener. Corn oil is a by-product of this process. Production of low erucic acid edible rapeseed oil has increased dramatically within the EEC due to financial incentives to producers (a subsidy of about \$200/tonne is currently being paid to farmers in the UK for rapeseed) and tariffs against imports to encourage indigenous oil production within the Community. Similar support has doubled production of sunflower oil to 1.1×10^6 MT within the EEC in the last 3 years.

It is ironic that soya oil should have such a dominant position in the oil market and be the benchmark against which an SCO process will be judged, since it was soya bean meal, after oil extraction, which effectively killed the embryonic SCP industry in Europe and elsewhere in the early 1970's which had predicted that the price of animal feedstuffs based on soya meal would increase, whereas it has decreased in real terms due to improved varieties, better agricultural practices and greater efficiency by the US producers.

The extracted meal from most oil seeds also has considerable value, because of it's protein content, as an animal feedstuff. The price of an oil therefore can reflect both it's own intrinsic value and the value of the residual meal or vice versa, depending on the state of the markets for both commodities. Soya beans contain only 18-20% oil, therefore most of their value lies in the quality and price of the meal, whereas rapeseed, peanuts, sunflower and cotton seed contain 30-40% oil, with oil a more important component of their price structure. Prices for oils in the 84/85 trading year were at similar levels to those of 78/79, after a dip in 82/83 and a substantial increase in 83/84 due to a drought in the U.S.A. and reduced supplies of Malaysian palm oil.

The oils and fats market is therefore a complex interaction between market forces for oils and oil seed meals, the weather in various parts of the world, changing agricultural practices, consumer demands and political intervention.

It is by no means a static marketplace, in the sense that new commodities are continually being introduced. The most spectacular example has been the growth of low erucic acid rapeseed (LEAR) production in Canada and Europe. Between 1971 and 1981 total world production of LEAR increased by 93%, over twice that of oils and fats in general. The original high erucic (HEAR), high glucosinolate rapeseed varieties were unsuitable for food use, but plant breeding has effectively produced an entirely new commodity. Production of rapeseed in the UK has grown from 38×10^3 MT in 1975, (high erucic varieties) to an estimated 950 x 10^3 MT in 1985/86, mainly the new LEAR varieties. It is predicted that world exports of oils and fats will rise by 32% in the second half of this decade, principally palm and LEAR oils.

Similar genetic selection has produced high oleic acid sunflower oil with better oxidative stability than the parent varieties. The new varieties have 80-85% oleic and <10% linoleic compared with 20-30% and 60-70% respectively. Sunflower strains are now available which crop well in Northern Europe which could lead to rapid development of this crop in areas with no previous history of oilseed production, in the same way as rapeseed has developed in Canada and Europe.

The fatty acid composition of the main oils and fats is shown in Table 2. The top 5 oils in terms of annual production, comprising 60% of the total, are composed principally of 16 and 18 carbon fatty acids with variable degrees of unsaturation of the 18 C family. Apart from palm oil, these oils are predominantly unsaturated 18 C fatty acids.

The higher priced edible oils such as olive oil and cocoa butter have special characteristics which make them valuable in their particular application. Production of both is small in terms of the major oils, 1.65 x 10^6 MT annually for olive oil and 0.8 x 10^6 MT for cocoa butter at a price of $2,000 and $3,6000/MT respectively. Olive oil is high in oleic acid (≅85%) but is otherwise not very different from the other major plant oils, except in its flavour characteristics. Commercial edible oils are refined and deodorised to produce a bland product, whereas both olive oil and some cocoa butters impart desirable flavour notes to the products in which they are incorporated as they are used without refining and deodorisation. These flavour characteristics would not be present in products of identical chemical composition from another source, such as a microbial oil.

Cocoa butter is one of the most expensive edible fats, costing between 2.2 and 2.4 times the price of cocoa beans which, like other commodities, fluctuates quite dramatically. At the beginning of 1985 the price of cocoa butter was $6,300/MT, whereas in March 1986 the price was $3,600/MT. The price of cocoa butter from 1979 to the end of February 1987 is shown in Fig. 1.

Table 2 Fatty acid compostion of principal commercial oils and fats.

Fatty acid compostition % w/w

Oil or Fat	12:0	14:0	16:0	18:0	18:1	18:2	18:3	20:0	20:2	22:0	22:1
Soya oil	-	-	11.0	4.0	22.0	53.0	7.5	1.0	1.0	-	-
Palm oil	0.1	1.0	45.0	4.5	37.7	10.6	0.2	0.3	_	-	-
Sunflower oil	-	0.1	5.5	4.7	19.5	68.5	0.1	-	-	-	-
Rapeseed LEAR	-	-	4	2	56	26	10	-	_	-	-
" LEAR	-	-	3	1	16	14	10	-	-	-	49
Cottonseed oil	-	0.8	27.3	2.0	18.3	50.5	-	0.3	-	-	-
Groundnut oil	-	-	12.5	2.5	37.9	41.1	0.3	0.5	0.7	2.5	1.0
Coconut oil	48.8	14.8	6.9	2.0	4.5	1.4	-	0.1	-	-	-
Olive oil	-	_	10.3	2.3	78.1	7.3	0.6	0.4	0.3	-	-
Palmkernel oil[a]	52.0	16.0	6.0	2.0	10.0	1.5	-	-	-	-	-
Corn oil	0.1	0.2	13.0	2.5	30.5	52.0	1.0	0.5	0.2	-	-
Cocoa butter	-	0.1	26.0	34.4	34.8	3.0	0.2	1.0	0.2	-	-
Linseed oil	-	-	6.1	3.2	16.6	14.2	59.8	-	-	-	-
Castor oil [b]	-	-	1.0	1.0	3.0	4.0	-	-	-	-	-
Tung oil [c]	-	-	3.1	2.1	11.2	44.4	11.4	-	-	-	-
Butter fat	2.4	9.0	22.1	14.3	30.4	1.2	2.6	-	-	-	-
Fish (herring)[d]	-	6.6	15.2	1.5	18.5	0.3	-	-	13.2	-	11.0

a 0.5% 6:0, 6.0% 8:0,5.0% 10:0.

b 90.0%, 12(OH), 18:1

c 69.0%, cis 9, trans 11, trans 13, 18:3.

d 13.3%, 20:5.

Data from Gunstone, Harwood & Padley, 1986.

Figure 1 Price of cocoa butter from 1979 - 1987.

Solid fats, such as cocoa butter, make an important contribution to the physical structure of products in which they are incorporated, related to both the fatty acid and triglyceride composition of the lipid. Edible oils which are liquid at ambient temperatures make relatively little contribution as their solid fat index (SFI), that is the proportion of solid to liquid fat at any temperature over the ambient range from 10-40°C is 0, whereas a hard fat such as cocoa butter shows a sharp melting point at about 35°C and the SFI increases rapidly. Plastic fats such as butter show a more gradual increase in SFI.

This property is what makes cocoa butter unique and hence valuable and is a function of the presence of an unusually high proportion of saturated fatty acids (>60%) for a vegetable fat together with a relatively uniform triglyceride composition where >70% of the triglyceride is composed of saturated fatty acids in the 1 and 3 positions and an unsaturated fatty acid in the 2 position. Butter has a much wider spread of triglycerides, and a less well defined melting point, whereas an oil like cottonseed oil composed of mainly triunsaturated triglycerides is liquid at all temperatures above 10°C.